THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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AUGUST TO DECEMBER, 1932

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Volume LXIII

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Preparation of Manuscript. In addition to the text matter, manuscripts should include a running page head of not more than thirty-five letters. Footnotes, tables, and legends for figures should be typed on separate sheets.

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THE MARINE BIOLOGICAL LABORATORY

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FORTY-FOURTH YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio,* who shall be the

President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is submitted my report as Treasurer of the Marine Biological Laboratory for the year 1931.

The accounts have been audited by Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1931, the book value of the General Endowment Fund in the hands of the Central Hanover Bank and Trust Company (of New York) as Trustee was $908,895 in securities and $53.77 in cash.

The book value of the Library Fund was $198,605 in securities, and $340 in cash.

The Reserve Fund consisted of securities of the book value of $20,868.75 and cash of $909.75.

The Retirement Fund consisted of securities of the book value of $18,896.07, invested in mortgages.

There has been little change in the other minor funds.

At this date (March 28, 1932) there is no default in any of the securities held in the above-mentioned funds.

The land, buildings, equipment, and library, excluding the Devil's Lane and Gansett properties, represented an investment of $1,642,665.24, less depreciation of $286,404.20, or a net amount of $1,356,261.04.

Current income exceeded expenses including depreciation by $2,304.35.

Over $21,000 was expended from current funds on buildings, equipment, and on books, the greater part having been expended for books.

At the end of the year, the Laboratory owed $2,349.65 in accounts payable, and $27,000 on bond and mortgage, and had over $30,000 in its bank accounts.

Following is the Balance Sheet as of December 31, 1931, and the condensed statement of income and outgo for the year, also the surplus account.

EXHIBIT A

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1931

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank & Trust Company (of New York), Trustee—Schedules I-a and I-b		$1,107,893.77	
Securities and Cash—Minor Funds—Schedule II		10,411.70	$1,118,305.47
Plant Assets:			
Land—Schedule IV	$ 97,103.05		
Buildings—Schedule IV	1,207,554.14		
Equipment—Schedule IV	162,965.48		
Library—Schedule IV	175,042.57	$1,642,665.24	
Less Reserve for Depreciation		286,404.20	
		$1,356,261.04	
Securities and Cash in Reserve Fund		21,778.50	
Cash in Dormitory Building Fund		818.96	$1,378,858.50
Current Assets:			
Cash		$ 30,872.07	
Accounts—Receivable		17,998.23	
Inventories:			
Supply Department	$ 36,327.34		
Biological Bulletin	9,077.28	45,404.62	
Investments:			
Devil's Lane Property	$ 39,301.81		
Gansett Property	1.00		
Stock in General Biological Supply House, Inc.	12,700.00		
Retirement Fund Assets	18,896.07	70,898.88	
Prepaid Insurance		3,902.12	
Items in Suspense (Net)		292.12	$ 169,368.04
			$2,666,532.01

Liabilities

Endowment Funds:			
General Endowment Funds—Schedule III		$1,107,893.77	
Minor Funds—Schedule III		10,411.70	$1,118,305.47
Plant Funds:			
Donations and Gifts—Schedule III		$1,029,372.61	
Other Investments in Plant from Gifts and Current Funds		347,485.89	
		$1,376,858.50	
Mortgage, Danchakoff Estate		2,000.00	$1,378,858.50

Current Liabilities and Surplus:			
Mortgage, Devil's Lane Property		$ 25,000.00	
Accounts—Payable		2,349.65	
Woods Hole Oceanographic Institution:			
Amount received for Purchase of Books for their Library	$ 5,000.00		
Less Expenditures	4,327.63	672.37	
		$ 28,022.02	
Current Surplus—Exhibit C		141,346.02	$ 169,368.04
			$2,666,532.01

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1931

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 47,293.49		$ 47,293.49
Library Fund		10,434.77		10,434.77
Gifts		445.29		445.29
Instruction	8,110.79	9,425.00		1,314.21
Research	4,063.36	18,085.00		14,021.64
Evening Lectures	167.58		167.58	
Biological Bulletin and Membership Dues	9,060.65	9,976.43		915.78
Supply Department—Schedule V	44,834.46	57,979.24		13,144.78
Mess—Schedule VI	30,664.25	34,360.65		3,696.40
Dormitories—Schedule VII	31,739.30	13,500.86	18,238.44	
(Interest and Depreciation charged to above 3 Departments. See Schedules V, VI, and VII)	*35,453.91*			35,453.91
Dividends, General Biological Supply House, Inc.		2,032.00		2,032.00
Rent, Danchakoff Cottages	505.17	1,039.00		533.83
Rent, Microscopes		469.00		469.00
Rent, Garage, Railway, etc.		427.60		427.60
Rent, Newman Cottage	92.94	150.00		57.06
Rent, Janitor's House	118.83	390.00		271.17
Sales of Duplicate Library Sets		238.70		238.70
Interest on Bank Balances		213.76		213.76
Sundry Items		71.82		71.82
Maintenance of Plant:				
New Laboratory Expense	18,057.74		18,057.74	
Chemical and Special Apparatus	9,869.52		9,869.52	
Maintenance, Buildings and Grounds	9,147.32		9,147.32	

Library Department Expenses	8,982.70		8,982.70	
Carpenter Department Expenses	1,602.02		1,602.02	
Truck Expenses	942.02		942.02	
Sundry Expenses	282.91		282.91	
Workmen's Compensation Insurance	554.02		554.02	
General Expenses:				
Administration Expenses	15,907.89		15,907.89	
Endowment Fund Trustee	968.50		968.50	
Interest on Loans	100.00		100.00	
Bad Debts	631.24		631.24	
Naples Zoological Station	250.00		250.00	
Mosquito Fund Contribution	100.00		100.00	
Reserve for Depreciation	39,778.56		39,778.56	
Museum Expenses	3,150.40		3,150.40	
Excess of Income over Expenses carried to Current Surplus—Exhibit C	2,304.35		2,304.35	
	$206,532.61	$206,532.61	$131,035.21	$131,035.21

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT
YEAR ENDED DECEMBER 31, 1931

Balance, January 1, 1931		$119,401.09
Add:		
Reserve for Depreciation charged to Plant Funds		39,778.56
Excess of Income over Expenses for Year as shown in Exhibit B		2,304.35
Excess of Gansett Property Receipts over Cost of Property and Development Expenses, etc.		1,885.95
Income of Retirement Fund	$ 734.47	
Less Pensions Paid	720.00	14.47
		$163,384.42
Deduct:		
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV,		
Buildings	$ 200.11	
Equipment	3,919.20	
Library Books, etc.	17,635.22	
	$21,754.53	
Income and Reserve Fund for 1931 credited to Current Surplus and now transferred to Plant Funds	283.87	22,038.40
Balance, December 31, 1931—Exhibit A		$141,346.02

Respectfully submitted,

LAWRASON RIGGS, JR.,

Treasurer.

V. THE REPORT OF THE LIBRARIAN

The expenditures of the Library remain the same as last year, $24,000 for the Marine Biological Laboratory, and $2,123.21 for the Oceanographic Institution. The $5,000 appropriated by the last-named in March, 1930, will be expended before the end of their fiscal year of February 29, 1932, for necessary books and back sets. A separate rendering of this account is kept on file. The only item in the regular $24,000 necessary to mention is that for current serials, which has, during 1930 and 1931, overreached the $5,000 assigned in 1929, by $72.94 and $631.07 respectively. In 1932, $6,000 is allowed for current serials. This increase was, however, anticipated in 1929 and the definite plan made to enlarge the subscriptions each year by a sum taken from the $8,200 assigned in 1929 for back sets. Until the back sets of 450 different serials are purchased which are very necessary to the Library before it is on a par with the finest libraries of the country, it is the judgment of the Librarian that the current expenditure on serials should not greatly exceed $6,000, thus leaving for a number of years the $7,000 that will, if wisely spent in completing back sets, increase the usefulness and monetary value of the Library much beyond the intrinsic sum spent. The full list of over 1,000 serial sets imperfect in the back holdings or in current receipts has been carefully scrutinized during the winter, 1931–32, and definite decision has been made, based on various different and combined reasons, to borrow or to leave unfilled about half of the number. Since every serial title in the Library, regardless of the method of acquisition and of its value, has been automatically recorded when deficient, the weeding out of half of these seems reasonable when choice was made without regard to any definite number to be retained or discarded, but the decisions are subject, of course, to future revision on the part of the investigators. Before the date of the summer report an estimate will have been made of the definite sum of money that will be necessary in order that the Library shall record in its catalogue 1,319 complete serial publications as against 869 perfectly complete now. A sum of $1,000 should always be available under this item, however, since new current serials will come on our list entailing the purchase in most cases of previously issued volumes.

A statement of the holdings of the Library shows the following: 1,080 currently received serials; 33,780 volumes; and 69,851 reprints. A detail of the current serials, 391 subscriptions, 481 exchanges, 208 gifts, shows an increase over last year in current paid subscriptions by 45, of which 14 were for the Woods Hole Oceanographic Institution, and 39 new exchanges. The low total increase from the year 1930 to

1931 from 1,060 to 1,080 is due to a rigid elimination of gifts and exchanges not regularly received, and will be enhanced another year when a selection will have been made of those important to us. As a temporary measure, a great number of incomplete and irregularly received United States Government publications have been entirely segregated from the serials, pending necessary decision as to their value for us.

The number of bound volumes added to the Library in 1931 was 1,923; of these 1,576 were serials, 48 sets having been filled in, seven of these for the Oceanographic, 33 partially filled in, two of these for the Oceanographic, and 347 were books. The unusual number of 156 books was purchased by us, using, in addition to the budgeted $300, a sum of $500 from the "back sets" money, and 95 were purchased for the Oceanographic Institution. In calling attention to the 33,780 volumes in the Library at the present time, it will not be out of place to record the fact that the accessioned number of volumes is a misleading figure as to the actual number of serial volumes the Library contains. For the sake of economy in binding and in order to coördinate thickness and height in volumes and make them look well as they stand on the shelves, we have during the past ten years increased rather than diminished the number in which we bind two or more volumes in one, thus reducing consistently the accessioned number below the actual. The shelf space occupied by the volumes indicates, indeed, a figure much more nearly 40,000 than the correct 33,780. The estimated capacity of the shelving space in the Library as given by the architect at 100,000 is correct for the small size volumes of our serial sets. Since one of the five floors of stacks must be reserved for reprints, the inference may be made that the space for serials and books will be completely exhausted only when the number 33,780 volumes, bound and accessioned in accordance with our present method, is doubled.

The reprints added to the files this year were 5,620; 2,745 of these were catalogued from the gift of Professor Metcalf and the Sidney I. Smith collection, and 2,875 total the year's current receipts. The majority of current reprints are from the authors, but the "Collected Papers" of 63 different laboratories are regularly received. Of these sets of reprints, 27 are complete and 36 are incomplete in the back files If these were counted as serial publications our list of current serial receipts would be 1,143 instead of 1,080. Of the reprints 1,828 are bound. Three hundred and seventy-five of these volumes contain 10–40 separates.

While the gifts of books from publishers are many fewer than some years ago when special effort was made to secure presentation copies direct from the publisher, the number from authors was exceptionally

high. This was due to Professor Baitsell and to Mr. Ware Cattell, who very generously presented to the Library the books reviewed during the summer in the "Collecting Net." A book plate records the gift as a joint one from the author, publisher, reviewer, and the "Collecting Net." The books presented to the Library this year are briefly enumerated below. These gifts are acknowledged with pleasure, and with very warm thanks.

"Collecting Net"	19
Authors	14
P. Blakiston's Son and Co.	4
Bruce Publishing Co.	1
Chicago University Press	1
Harvard University Press	1
John Wiley & Sons, Inc.	1
Alfred A. Knopf	1
Lea & Febiger	1
Macmillan Co.	2
W. B. Saunders & Co.	1
Dr. Henry McE. Knower	3
Dr. C. A. Cheever	1
National Academy of Sciences	1
Eastman Kodak Company	1

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-fourth session of the Marine Biological Laboratory for the year 1931.

1. *Attendance.* The attendance for 1931 was unexpectedly large, the total number of investigators for the season being 362 as compared with 337 in 1930, the next-highest year. The tabular view of attendance on page 29 shows in detail the relation of the past season to the four immediately preceding it. Particularly noteworthy is the large number of institutions represented in 1931, this being 137 as compared with 126 in 1930 and 123 in 1929.

Of considerable importance to the Laboratory from a practical standpoint is the peak attendance. It is this, rather than the total attendance, which determines the degree of crowding of the Laboratory, the Mess, and the available living accommodations. Following the high peak of 286 in 1928, a measure of relief was afforded for a time by a new arrangement of the courses, which tended to spread the attendance over a longer period; and, for several years, a further growth of the total attendance occurred without evidences of undue crowding. During the past season, however, the peak attendance suddenly increased by 43 over that for 1930 and surpassed by 20 even the previous high

record of 1928. Since it seems impracticable at present for the Laboratory to increase the available research and living accommodations, the attention of investigators is again invited to the advantages of utilizing to a greater extent than at present the early and the late parts of the season. The following tabulation of attendance on selected dates for the past five years gives a clear view of the general situation.

		1927	1928	1929	1930	1931
May	30	7	15	9	6	6
June	10	50	64	55	50	51
"	20	114	140	139	153	153
"	30	212	240	197	208	217
July	10	247	281	238	253	258
"	20	247	282	242	250	273
"	30	245	272	249	253	281
August	10	234	250	256	254	302
"	20	208	226	243	245	280
"	30	168	183	220	204	239
September	10	110	112	157	122	136
"	20	50	43	59	44	69
"	30	12	14	14	8	14

2. *The Report of the Treasurer.* This report shows a slight increase in the total assets of the Laboratory over the preceding year, the figures being $2,666,532.01 for 1931 as compared with $2,660,559.11 for 1930. Though the income for 1931 was less than that for 1930 ($206,532.61 as compared with $210,110.86), a reduction of expenses from $213,878.11 to $204,228.36 permitted for the first time in six years the appearance of an excess of income over expenses, including depreciation charges. While the Laboratory was not greatly affected during 1931 by the existing financial depression, the indications at the time of the preparation of this report are that the year 1932 will show a serious decrease in the subscriptions received from coöperating and subscribing institutions and somewhat smaller decreases in the income from the Supply Department and from the permanent endowment fund. It is hoped, however, that by means of all practicable economies it may be possible to preserve the very gratifying condition of financial soundness that has characterized the Laboratory for so many years, even though the exceptionally favorable showing for 1931 may not again be equalled for some time.

3. *The Report of the Librarian.* The growth of the Library in 1931 has continued at approximately the same rate as that for the past six years. Its development since 1925 may perhaps best be shown by means of the following figures taken in part from the reports of previous years.

Particularly noteworthy is the fact that 869 sets of serial publications are now complete; these include most of the sets in common use.

	1925	1926	1927	1928	1929	1930	1931
Serials received currently	500	628	764	874	985	1060	1080
Total number of bound volumes	15000	18200	22800	26500	28300	31500	33800
Reprints	25000	38000	43000	51000	59000	64000	70000

4. *Publications.* During the early years of the Laboratory, a record was kept of all the published scientific work that issued from it. With its continued growth, however, the difficulty of keeping such a record became very great and the practice was discontinued about twenty years ago. The development in recent years of a trained library staff accustomed to the collection and the cataloguing of reprints has again made it possible, not merely to keep a record of all publications, but to bind and index the papers themselves in such a way that they may be readily available both for the use of investigators and of other persons interested in the scientific accomplishments of the Laboratory. Through the kind coöperation of the investigators concerned, approximately 100 papers, based on work done at the Laboratory and published in 1930, are now ready for binding. It is planned to prepare similar sets for each subsequent year; and it is to be expected that the value of these sets, both historical and scientific, will in time become very great. The thanks of the Laboratory are due to all who have so generously contributed the necessary reprints of their papers.

5. *Lectures and Scientific Meetings.* Twelve evening lectures were delivered during 1931, including the special Reynold A. Spaeth Memorial Lecture by Professor Ross G. Harrison. In addition, there were held 12 other meetings, at which 63 shorter scientific papers were presented and discussed.

A successful innovation in 1931 was the special scientific session held on September 3 and devoted exclusively to work completed at the Laboratory during the current season. Twenty-two papers conveniently grouped by subjects were presented at this session, which occupied the greater part of the day. The remainder of the day was devoted to the inspection of demonstrations of work in progress at the Laboratory. The titles of the various lectures and shorter papers for 1931, which give a very representative cross-section of the work of the Laboratory for that year, are listed on pages 31 to 36.

6. *Supply Department and Museum.* The period of management of the Supply Department by the General Biological Supply House

having terminated in August, 1931, Mr. James McInnis, who for the past year had acted as Resident Manager, was placed in full charge of the Department. Under his management the Supply Department has continued, in spite of generally unfavorable business conditions, to provide an important part of the revenue needed for the running of the Laboratory, besides filling with efficiency its primary function of supplying living material to investigators.

During the past year very gratifying progress has been made in the development of a working museum by its Curator, Mr. George M. Gray. A large number of representatives of the local fauna, properly preserved and labeled, are now available for the use of investigators who wish to identify material of their own or whose work in other ways requires museum facilities. In addition to the preservation of material, particular attention has been given during the year to the accumulation and tabulation of data on the geographical and seasonal distribution of the local forms. Mr. Gray's long experience with the fauna of the Woods Hole region gives him exceptional qualifications for carrying out this very important part of the work of the Museum.

7. *Meeting of the Corporation.* At the annual meeting of the Corporation, held on August 11, the report of a special committee appointed a year previously to consider changes in the method of nominating trustees was considered, and the following recommendations of the Committee were adopted:

1. "The Corporation affirms its position that instruction is a fundamental part of the work of the Laboratory and hence this work should be adequately represented upon the Board of Trustees."
2. "That the Committee of the Corporation for nomination of Trustees consist of five members, of whom not less than two shall be Trustee members and not less than two shall be non-Trustee members of the Corporation."
3. "That on or about July first of each year, the Clerk shall send a circular letter to each member of the Corporation giving the names of the Nominating Committee and stating that this Committee desires suggestions regarding nominations."
4. "That the Nominating Committee shall post the list of nominations at least one week in advance of the annual meeting of the Corporation."

A recommendation that no trustee shall be eligible for re-election until one year after the expiration of the term for which he was elected was discussed at length, but the motion to accept it was lost. The following new trustees were elected by the Corporation: L. V. Heilbrunn (Class of 1935), H. B. Goodrich (Class of 1933).

Following the request of Dr. Gary N. Calkins, who for 19 years had ably served the Laboratory as Clerk of the Corporation, that his name be not presented to the Corporation for re-election, the Nominating Com-

mittee selected in his place Dr. Charles Packard, of Columbia University, who was duly elected to the position, thereby becoming at the same time a Trustee ex-officio. Dr. Calkins, who on his retirement from office ceased to be a Trustee ex-officio, was elected to fill one of the regular positions in the Class of 1935. A resolution of appreciation of the long and valued services of Dr. Calkins as its Clerk was adopted by a rising vote of the Corporation.

8. *Changes in the By-laws.* At the annual meeting of the Board of Trustees two changes in the By-laws were approved. Those parts of the By-laws affected by the changes in question now read, as amended:

1. "The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11:30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years."

2. "Trustees ex efficio and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote."

9. *Gifts.* Appreciative acknowledgment is made of the assistance of the Committee on the Effects of Radiation of the National Research Council and of various persons, who through the efforts of this Committee became interested in the needs of the Laboratory, in connection with investigations requiring the use of X-rays and other types of radiation. Without this assistance much of the work accomplished in this field during the past year would have been impossible. The thanks of the Laboratory are also due to Mrs. J. C. Hemmeter for the gift of scientific apparatus formerly belonging to her husband, the late Dr. John C. Hemmeter, and to Mr. Ware Cattell for the continuation in 1931 of the Collecting Net Scholarships and for the gift to the Library of a considerable number of books.

There are appended as parts of this report:

1. The Staff, 1931.
2. Investigators and Students, 1931.
3. A Tabular View of Attendance, 1927–1931.
4. Subscribing and Coöperating Institutions, 1931.
5. Evening Lectures, 1931.
6. Shorter Scientific Papers, 1931.
7. Members of the Corporation, August, 1931.

Respectfully submitted,

M. H. Jacobs,
Director.

1. THE STAFF, 1931

MERKEL H. JACOBS, *Director*, Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
E. G. CONKLIN, Professor of Zoölogy, Princeton University.
CASWELL GRAVE, Professor of Zoölogy, Washington University.
H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.
FRANK R. LILLIE, Professor of Embryology, University of Chicago.
C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.
S. O. MAST, Professor of Zoölogy, Johns Hopkins University.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoölogy, Harvard University.
E. B. WILSON, Professor of Zoölogy, Columbia University.
LORANDE L. WOODRUFF, Professor of Protozoölogy, Yale University.

II. INSTRUCTION

J. A. DAWSON, Assistant Professor of Biology, College of the City of New York.
T. H. BISSONNETTE, Professor of Biology, Trinity College.
E. C. COLE, Associate Professor of Biology, Williams College.
O. E. NELSEN, Instructor in Zoölogy, University of Pennsylvania.
A. W. POLLISTER, Instructor in Zoölogy, Columbia University.
L. P. SAYLES, Instructor in Biology, College of the City of New York.
A. E. SEVERINGHAUS, Assistant Professor of Anatomy, College of Physicians and Surgeons, Columbia University.

JUNIOR INSTRUCTORS

B. R. COONFIELD, Professor of Biology, Southwestern College.
C. E. HADLEY, Assistant Professor of Biology, New Jersey State Teachers College at Montclair.

PROTOZOÖLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
RACHEL BOWLING, Instructor in Zoölogy, Columbia University.
W. BYERS UNGER, Assistant Professor of Zoölogy, Dartmouth College.

EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

Hubert B. Goodrich, Professor of Biology, Wesleyan University.
Benjamin H. Grave, Professor of Biology, De Pauw University.
Leigh Hoadley, Professor of Zoölogy, Harvard University.
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
Harold H. Plough, Professor of Biology, Amherst College.

PHYSIOLOGY

I. Investigation

Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

William R. Amberson, Professor of Physiology, University of Tennessee.
Philip Bard, Assistant Professor of Physiology, Harvard Medical School.
Ralph W. Gerard, Assistant Professor of Physiology, University of Chicago.
Laurence Irving, Associate Professor of Physiology, University of Toronto.
Leonor Michaelis, Member of the Rockefeller Institute, New York City.
Margaret Sumwalt, Assistant Professor of Physiology, Woman's Medical College of Pennsylvania.

Special Lecturers

Edwin J. Cohn, Associate Professor of Physical Chemistry, Harvard University.
Henry J. Fry, Associate Professor of Biology, Washington Square College, New York University.
E. Newton Harvey, Professor of Physiology, Princeton University.
Selig Hecht, Professor of Biophysics, Columbia University.
Merkel H. Jacobs, Professor of General Physiology, University of Pennsylvania.
Balduin Lucké, Associate Professor of Pathology, University of Pennsylvania.

BOTANY

I. Investigation

B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Pennsylvania.
Hannah T. Croasdale, Biological Abstracts, University of Pennsylvania.
James P. Poole, Professor of Evolution, Dartmouth College.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Hester Ann Bradbury, Hazel Blanchard, Mary A. Rohan, Assistants.

CHEMICAL SUPPLIES

Oscar W. Richards, Instructor in Biology, Yale University.

SCIENTIFIC APPARATUS AND TECHNICAL SUPPLIES

Samuel E. Pond, Assistant Professor of Physiology, Schools of Medicine and Dentistry, University of Pennsylvania, in charge.

A. R. Apgar, Photographer.
J. D. Graham, Glassblower.
Lester F. Boss, Mechanician.
P. H. Liljestrand, Assistant.

MUSEUM

George M. Gray, Curator.

SUPPLY DEPARTMENT

James McInnis, Manager.
A. M. Hilton, Collector.
Milton B. Gray, Collector.
Walter Kahler, Collector.
Geoffrey Lehy, Collector.
A. W. Leathers, Shipping.

BOATS

John J. Veeder, Captain.
E. M. Lewis, Chief Engineer.

F. M. MacNaught, Business Manager.
Herbert A. Hilton, Superintendent of Buildings and Grounds.
Thomas Larkin, Superintendent of Mechanical Department.
William Hemenway, Carpenter.

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2. INVESTIGATORS AND STUDENTS, 1931

Independent Investigators

Adams, A. Elizabeth, Professor of Zoölogy, Mount Holyoke College.
Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Allee, W. C., Professor of Zoölogy, University of Chicago.
Amberson, William R., Professor of Physiology, University of Tennessee.
Anderson, Rubert S., Research Associate, Princeton University.
Armstrong, Philip B., Assistant Professor of Anatomy, Cornell University Medical College.

ASTROM, I. ELISABETH, Class Assistant, University of Toronto.
AUSTIN, MARY L., Instructor in Zoölogy, Wellesley College.
BAILEY, PERCY L., JR., Instructor in Physiology, College of the City of New York.
BAITSELL, GEORGE A., Professor of Biology, Yale University.
BAKWIN, HARRY, Assistant Clinical Professor, New York University.
BAKWIN, RUTH MORRIS, Instructor in Pediatrics, New York University.
BALL, ERIC G., Instructor in Physical Chemistry, Johns Hopkins University Medical School.
BALLARD, WILLIAM W., Instructor in Zoölogy, Dartmouth College.
BARD, PHILIP, Assistant Professor of Physiology, Harvard Medical School.
BARRÓN, E. S. GUZMAN, Research Associate, University of Chicago.
BARTH, L. G., National Research Council Fellow, University of Chicago.
BEAMS, H. W., Assistant Professor of Zoölogy, State University of Iowa.
BELKIN, MORRIS, Instructor in Biology, Washington Square College, New York University.
BEUTNER, R., Professor of Pharmacology, University of Louisville, School of Medicine.
BISSONNETTE, THOMAS H., Professor of Biology, Trinity College.
BODANSKY, OSCAR, Instructor in Pediatrics, New York University and Bellevue Hospital.
BORODIN, D. N., 621 West 42d Street, New York City.
BOWLING, RACHEL, Instructor in Zoölogy, Columbia University.
BRADLEY, H. C., Professor of Physiological Chemistry, University of Wisconsin.
BRIDGES, CALVIN B., Research Assistant in Genetics, Carnegie Institution of Washington.
BRINLEY, FLOYD J., Assistant Professor of Zoölogy, North Dakota State College.
BRONFENBRENNER, J., Professor of Bacteriology and Public Health, Washington University Medical School.
BUDINGTON, ROBERT A., Professor of Zoölogy, Oberlin College.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CANNAN, ROBERT K., Professor of Chemistry, New York University and Bellevue Hospital Medical College.
CARPENTER, RUSSELL L., Instructor in Anatomy, College of Physicians and Surgeons, Columbia University.
CARVER, GAIL L., Professor of Biology, Mercer University.
CASTLE, WILLIAM A., Instructor in Biology, Brown University.
CATTELL, WARE, New York University.
CHAMBERS, ROBERT, Research Professor and Chairman of Department of Biology, Washington Square College, New York University.
CHEEVER, CLARENCE A., Member, Boston Society of Natural History.
CHENEY, RALPH H., Chairman, Biology Department, Long Island University.
CHIDESTER, F. E., Professor of Zoölogy, West Virginia University.
CHRISTIE, JESSE R., Associate Nematologist, United States Department of Agriculture.
CLARK, ELEANOR LINTON, Research Assistant in Anatomy, University of Pennsylvania.
CLARK, ELIOT R., Director of Department of Anatomy, University of Pennsylvania.
CLOWES, G. H. A., Director, Lilly Research Laboratories.
COBB, N. A., Principal Nematologist, United States Department of Agriculture.
COE, WESLEY R., Professor of Biology, Yale University.
COLE, ELBERT C., Associate Professor of Biology, Williams College.
COLE, KENNETH S., Assistant Professor of Physiology, College of Physicians and Surgeons, Columbia University.
CONKLIN, EDWIN G., Professor of Biology, Princeton University.
COONFIELD, B. R., Instructor in Zoölogy, Brooklyn College of the City of New York.

COPELAND, MANTON, Professor of Biology, Bowdoin College.
COWDRY, E. V., Professor of Cytology, Washington University.
COWLES, R. P., Professor of Zoölogy, Johns Hopkins University.
CURTIS, W. C., Professor of Zoölogy, University of Missouri.
CURWEN, ALICE O., Instructor in Histology and Embryology, Woman's Medical College of Pennsylvania.
DANKS, W. B. C., Government Officer of Kenya Colony.
DARRAH, WM. C., Fellow in Paleobotany, Carnegie Museum, University of Pittsburgh.
DAWSON, ALDEN B., Associate Professor of Zoölogy, Harvard University.
DAWSON, J. A., Assistant Professor of Biology, College of the City of New York.
DODDS, GIDEON S., Professor of Histology and Embryology, West Virginia University.
DOLLEY, WILLIAM L., JR., Professor of Biology, University of Buffalo.
DONALDSON, HENRY H., Member, Wistar Institute.
DUBOIS, EUGENE F., Professor of Medicine, Cornell University Medical College.
DUNBAR, FRANCIS F., Graduate Assistant in Zoölogy, Columbia University.
EDWARDS, DAYTON J., Associate Professor of Physiology, Cornell University Medical College.
EINARSON, LARUS, Research Fellow of the Rockefeller Foundation, Harvard University Medical School.
FAILLA, G., Physicist, Memorial Hospital, New York City.
FAVILLI, GIOVANNI, First Assistant in the Institute of General Pathology, Royal University, Florence, Italy.
FOGG, LLOYD C., Instructor in Biology, Washington Square College, New York University.
FRASER, DORIS A., Assistant in Anatomy, University of Pennsylvania.
FRENCH, CHARLES S., Graduate Student and Assistant in General Physiology, Harvard University.
FRY, HENRY J., Professor of Biology, Washington Square College, New York University.
FURTH, JACOB, Associate in Pathology, The Henry Phipps Institute.
GARREY, W. E., Professor of Physiology, Vanderbilt University School of Medicine.
GAYET, RENÉ, Directeur Adjoint Laboratoire de Physiologie Pathologique, Collège de France.
GEIMAN, QUENTIN M., Director of Science Department, Swarthmore Preparatory School.
GELFAN, SAMUEL, Assistant Professor of Physiology and Pharmacology, University of Alberta.
GERARD, R. W., Associate Professor of Physiology, University of Chicago.
GILSON, LEWIS E., Instructor in Biochemistry, University of Cincinnati.
GOLDFORB, A. J., Professor of Biology, College of the City of New York.
GOODRICH, HUBERT B., Professor of Biology, Wesleyan University.
GRAVE, B. H., Professor of Zoölogy, DePauw University.
GRAVE, CASWELL, Professor of Zoölogy, Washington University.
GREEN, ARDA A., Research Fellow in Physical Chemistry, Harvard University Medical School.
GREENWOOD, ALAN W., Lecturer, Institute of Genetics, University of Edinburgh.
GRUNDFEST, HARRY, National Research Council Fellow, Johnson Foundation, University of Pennsylvania.
HADLEY, CHARLES E., Assistant Professor of Biology, New Jersey State Teachers College.
HAHNERT, WILLIAM F., National Research Fellow, The Johns Hopkins University.
HAM, ARTHUR W., Instructor in Cytology, Washington University School of Medicine.

HAMBURGER, RUDOLF T., Assistant in the Medical Clinic, University of Groningen, Holland.
HARNLY, MORRIS H., Assistant Professor, New York University.
HARTLINE, H. K., Fellow, Johnson Foundation, University of Pennsylvania.
HARVEY, ETHEL BROWNE, Assistant in Biology, Washington Square College, New York University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HAYDEN, MARGARET A., Assistant Professor of Zoölogy, Wellesley College.
HAYNES, FLORENCE W., Harvard University Medical School.
HAYWOOD, CHARLOTTE, Associate Professor of Physiology, Mount Holyoke College.
HEILBRUNN, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
HELWIG, EDWIN R., Instructor, University of Pennsylvania.
HENDERSON, JEAN T., Lecturer, McGill University.
HENSHAW, PAUL S., Biophysicist, Memorial Hospital, New York City.
HILL, SAMUEL E., Assistant in Physiology, Rockefeller Institute.
HOADLEY, LEIGH, Professor of Zoölogy, Harvard University.
HODGE, CHARLES, JR., Instructor in Zoölogy, University of Pennsylvania.
HODGE, RUTH M. PATRICK, University of Virginia, Charlottesville, Virginia.
HOGUE, MARY JANE, Instructor in Anatomy, Medical School, University of Pennsylvania.
HOOK, SABRA J., Instructor in Biology, University of Rochester.
HOPPE, ELLA N., Research Assistant, Division of Laboratories and Research, New York State Department of Health.
HORNING, E. S., University of Sydney.
HOWARD, EVELYN, University of Pennsylvania.
HOWE, H. E., Editor, Industrial and Engineering Chemistry.
HUETTNER, ALFRED F., Associate Professor, Washington Square College, New York University.
IMAI, TAKEO, Assistant in Biology, Tohoku Imperial University, Sendai, Japan.
IRVING, LAURENCE, Associate Professor of Physiology, University of Toronto.
IRWIN, MARIAN, Associate, Rockefeller Institute.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JOHLIN, J. M., Associate Professor of Biochemistry, Vanderbilt University, School of Medicine.
JOHNSON, DUNCAN S., Professor of Botany, Johns Hopkins University.
JOHNSON, H. HERBERT, Instructor, College of the City of New York.
JOHNSTON, ROBERT L., Head of Research Division, Cleveland Clinic Foundation.
KAUFMANN, BERWIND P., Professor of Botany, University of Alabama.
KEEFE, REV. A. M., Rector, St. Norbert College.
KEIL, ELSA M., Instructor in Zoölogy, New Jersey College for Women.
KILLE, FRANK R., Associate Professor of Biology, Birmingham-Southern College.
KINDRED, JAMES E., Associate Professor of Histology and Embryology, University of Virginia.
KING, ROBERT L., Associate Professor, State University of Iowa.
KIRBY-SMITH, HENRY T., Instructor in Anatomy, University of Pennsylvania.
KNOWER, HENRY MCE., Associate Professor of Anatomy, Albany Medical College.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University, College of Medicine.
KOSTIR, WENCEL J., Assistant Professor of Zoölogy, Ohio State University.
LACKEY, JAMES B., Professor of Biology, Southwestern.
LEVINE, PHILIP, Associate, Rockefeller Institute.
LEWIS, IVEY F., Professor of Botany, University of Virginia.
LILLIE, FRANK R., Chairman of the Department of Zoölogy, University of Chicago.
LILLIE, RALPH S., Professor of General Physiology, University of Chicago.
LOEBEL, ROBERT O., Fellow, Cornell University Medical College.
LUCAS, ALFRED M., Assistant Professor of Cytology, Washington University Medical School.

Lucas, Miriam Scott, Instructor in Cytology, Washington University Medical School.
Lucké, Balduin, Associate Professor of Pathology, University of Pennsylvania.
Lund, E. J., Professor of Physiology, University of Texas.
Lynch, Ruth Stocking, Instructor in Genetics, Johns Hopkins University.
McClung, C. E., Director, Zoölogical Laboratory, University of Pennsylvania.
McGlone, Bartgis, Instructor in Physiology, University of Pennsylvania.
McGoun, Ralph C., Jr., Instructor in Biology, Amherst College.
McGregor, James H., Professor of Zoölogy, Columbia University.
Margolin, Sydney, Graduate Student, Columbia University.
Mathews, Albert P., Carnegie Professor of Biochemistry, University of Cincinnati.
Mavor, James W., Professor of Biology, Union College.
Metz, Charles W., Professor, Johns Hopkins University.
Michaelis, L., Member, Rockefeller Institute for Medical Research.
Miller, Helen M., National Research Council Fellow, Johns Hopkins University.
Mitchell, Philip H., Professor of Physiology, Brown University.
Morgan, Ann H., Professor of Zoölogy, Mount Holyoke College.
Morgan, Lilian V., California Institute of Technology.
Morgan, T. H., Professor of Biology, California Institute of Technology.
Morgulis, Sergius, Professor of Biochemistry, University of Nebraska.
Morrill, Charles V., Associate Professor of Anatomy, Cornell University Medical College.
Morris, Helen S., Graduate Student, Columbia University.
Nabrit, S. Milton, Professor of Biology, Morehouse College.
Navez, Albert E., Lecturer in General Physiology, Harvard University.
Nelsen, Olin E., Instructor in Zoölogy, University of Pennsylvania.
Nicholas, Warren W., X-Ray Physicist, National Bureau of Standards.
Nonidez, José F., Assistant Professor of Anatomy, Cornell University Medical College.
Orr, Paul R., Instructor, University of Pennsylvania.
Osterhout, W. J. V., Member, Rockefeller Institute for Medical Research.
Packard, Charles, Assistant Professor of Zoölogy, Columbia University.
Papenfuss, George F., Student Assistant, Johns Hopkins University.
Parker, G. H., Professor of Zoölogy, Harvard University.
Parmenter, Charles L., Associate Professor of Zoölogy, University of Pennsylvania.
Parpart, Arthur K., Instructor in Physiology, University of Pennsylvania.
Patch, Esther M., Windsor, Vermont.
Payne, Fernandus, Head of Department of Zoölogy and Dean of Graduate School, Indiana University.
Payne, Nellie M., University of Pennsylvania.
Peebles, Florence, Professor of Biology, California Christian College.
Pinney, Mary E., Professor of Zoölogy, Milwaukee-Downer College.
Plough, Harold H., Professor of Biology, Amherst College.
Pollister, Arthur W., Instructor in Zoölogy, Columbia University.
Pollister, Priscilla Frew, Graduate Student, Columbia University.
Pond, Samuel E., Assistant Professor of Physiology, University of Pennsylvania.
Poole, James P., Professor of Evolution, Dartmouth College.
Raffel, Daniel, Fellow, Johns Hopkins University.
Redfield, Helen, California Institute of Technology.
Reese, Albert M., Head of Department of Zoölogy, West Virginia University.
de Renyi, George S., Associate Professor of Anatomy, University of Pennsylvania.
Reznikoff, Paul, Instructor in Medicine, Cornell University Medical College.
Richards, Alfred N., Professor of Pharmacology, University of Pennsylvania.
Richards, Oscar W., Instructor in Biology, Yale University.

RIJLANT, PIERRE, Professor of Human Physiology, University of Brussels.
RISLEY, PAUL L., Instructor in Zoölogy, University of Michigan.
ROOT, WALTER S., Assistant Professor of Physiology, College of Medicine, Syracuse University.
RUGH, ROBERTS, Instructor in Zoölogy, Hunter College.
SAYLES, LEONARD P., Instructor in Biology, College of the City of New York.
SCHAUFFLER, WILLIAM G., Princeton, New Jersey.
SCHMIDT, IDA T. GENTHER, Research Fellow, Children's Hospital, Cincinnati, Ohio.
SCHMIDT, LEON H., Research Fellow, University of Cincinnati.
SCHRADER, FRANZ, Professor of Zoölogy, Columbia University.
SCHRADER, SALLY HUGHES, Columbia University.
SCHULTZ, JACK, Investigator, Carnegie Institute of Washington.
SCOTT, SISTER FLORENCE MARIE, Assistant Professor, Seton Hill College.
SELLMEYER, BERNARD L., Head of Department and Professor of Biology, Loyola University.
SEVERINGHAUS, AURA E., Assistant Professor of Anatomy, Columbia University.
SICKLES, GRACE, Assistant Bacteriologist, New York State Department of Health.
SLIFER, ELEANOR H., National Research Council Fellow in Zoölogy, State University of Iowa.
SNOOK, THEODORE, Instructor in Histology and Embryology, Cornell University.
SONNEBORN, TRACY M., Research Associate, Johns Hopkins University.
SPEIDEL, CARL C., Associate Professor of Anatomy, University of Virginia.
SPEMANN, HANS, Travelling Professor, Rockefeller Foundation.
STEINBACH, HENRY BURR, Instructor in Zoölogy, University of Pennsylvania.
STEWART, DOROTHY R., Assistant Professor of Biology, Skidmore College.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell University Medical College.
STREET, SIBYL, 29 Jewett Place, Utica, New York.
STRONG, OLIVER S., Professor of Neurology and Neuro-Histology, Columbia University.
SUMWALT, MARGARET, Assistant Professor of Physiology, Woman's Medical College of Pennsylvania.
TAFT, CHARLES H., JR., Associate Professor of Pharmacology, University of Texas, Medical School.
TASHIRO, SHIRO, Professor of Biochemistry, University of Cincinnati.
TAYLOR, WM. RANDOLPH, Professor of Botany, University of Michigan.
TITTLER, IRVING A., Assistant in Zoölogy, Columbia University.
TITUS, CHARLES P., East Orange, New Jersey.
TOHYAMA, GUZO, Assistant Professor, Tokyo Imperial University.
TURNER, JOHN P., Instructor in Zoölogy, University of Minnesota.
TYLER, ALBERT, Instructor in Embryology, California Institute of Technology.
UNGER, W. BYERS, Assistant Professor of Zoölogy, Dartmouth College.
VAN SLYKE, E., Instructor, University of Pittsburgh.
VICARI, EMILIA M., Research Associate in Anatomy, Cornell University Medical College.
WALKER, RUTH I., Instructor in Botany, University of Wisconsin.
WARREN, HOWARD C., Professor of Psychology, Princeton University.
WHEDON, ARTHUR D., Professor of Zoölogy and Head of Department of Zoölogy and Physiology, North Dakota Agricultural College.
WHITAKER, D. M., Assistant Professor of Zoölogy, Columbia University.
WHITING, ANNA R., Professor of Biology, Head of Department, Pennsylvania College for Women.
WHITING, P. W., Associate Professor of Zoölogy, University of Pittsburgh.
WIEMAN, H. L., Professor of Zoölogy, University of Cincinnati.
WILSON, EDMUND B., Professor Emeritus, Columbia University.
WITSCHI, EMIL, Professor of Zoölogy, State University of Iowa

WODEHOUSE, ROGER P., Director of the Biological Laboratory, The Arlington Chemical Co.
WOLF, E. ALFRED, Assistant Professor of Zoölogy, University of Pittsburgh.
WOODWARD, ALVALYN E., Assistant Professor, University of Michigan.
YOUNG, ROGER ARLINER, Assistant Professor of Zoölogy, Howard University.

Beginning Investigators

ADAMS, EDGAR M., Laboratory Assistant, University of Cincinnati.
APGAR, GRACE M., University of Pennsylvania.
ATLAS, MEYER, Assistant in Embryology, Columbia University.
AURINGER, JACK, Graduate Student, Detroit City College.
BECK, LYLE V., Graduate Assistant in Physiology, New York University.
BENKERT, JOSEPH M., Science Director, Ambridge High School.
BENKERT, LYSBETH HAMILTON, Instructor, Pennsylvania College for Women.
BOSTIAN, C. H., Assistant Professor of Zoölogy, North Carolina State College.
BRADWAY, WINNEFRED, Assistant in Biology, Washington Square College, New York University.
BUCHHEIT, J. ROBERT, Graduate Assistant in Zoölogy, University of Illinois.
CABLE, RAYMOND M., Teaching Assistant, New York University.
CARLSON, J. GORDON, Demonstrator in Biology, Bryn Mawr College.
CHEN, T. T., Instructor and Graduate Student, University of Pennsylvania.
CHOR, HERMAN, Research Fellow, Washington University Medical School.
CLINE, ELSIE, Graduate Student, Johns Hopkins University.
COHEN, BERNARD M., Student, Johns Hopkins University.
CORSON, SAMUEL A., University of Pennsylvania.
COSTELLO, DONALD P., Instructor in Zoölogy, University of Pennsylvania.
CROASDALE, HANNAH T., Scientific Staff of Biological Abstracts, University of Pennsylvania.
DAN, KATSUMA, University of Pennsylvania.
DAUGHERTY, KATHRYN, Research Assistant, University of Pennsylvania.
DAVIS, J. F., Instructor in Zoölogy, University of Pennsylvania.
DEARING, WILLIAM H., Student, University of Pennsylvania Medical School.
EASTLICK, HERBERT L., Part Time Assistant, Washington University.
FIELD, MADELEINE E., Research Fellow in Physiology, Harvard School of Public Health.
GILMORE, KATHRYN, Graduate Student, University of Pittsburgh.
GRAY, NINA E., Assistant in Zoölogy, University of Wisconsin.
GREEN, DAVID E., Assistant, Washington Square College, New York University.
HAMBURGH, MORTON, Johns Hopkins University Medical School.
HEISS, ELIZABETH, Instructor in Histology, Purdue University.
HILL, EDGAR S., University of Cincinnati.
HITSCHLER, WILLIAM J., Research Worker, University of Pennsylvania.
ISHII, KENJIRO, Graduate Student, University of Cincinnati.
JACKSON, JOHN R., Graduate Assistant in Botany, University of Missouri.
JONES, EDGAR P., Instructor, University of Pittsburgh.
KATZ, JACOB D., Graduate Student, New York University.
KELTCH, ANNA K., Lilly Research Laboratories.
KIDDER, GEORGE W., Graduate Student, Columbia University.
KINNEY, ELIZABETH T., Assistant in Zoölogy, Barnard College.
KRAJNIK, BOHUMIL, Research Assistant, University at Bratislava, Czechoslovakia.
LAMBERT, ELIZABETH F., Technician, Harvard University Medical School.
LUNDSTROM, HELEN M., Graduate Student, University of Pennsylvania.
MCQUESTEN, BARBARA, 14 Still Street, Brookline, Massachusetts.
MACHELLA, THOMAS E., Assistant Instructor, University of Pennsylvania.
MANN, D. R., Graduate Assistant, Duke University.
MARSHAK, ALFRED, Student, Bussey Institution.

MARSLAND, DOUGLAS A., Assistant Professor of Biology, Washington Square University, New York University.
MEDARIS, DON, Student, DePauw University.
MELTZER, ADOLPH, Student, Cornell University Medical College.
MENDELSON, EMANUEL S., Research Technician, University of Pennsylvania.
MICHAELIS, EVA, 1185 Park Avenue, New York City.
MORRIS, SAMUEL, Instructor in Zoölogy, University of Pennsylvania.
NICOLL, PAUL A., Part Time Assistant, Washington University.
OLTMANN, CLARA, Student, Columbia University.
ORMSBY, ANDREW A., 14209 Winthrop Avenue, Detroit, Michigan.
PARKS, MARK E., Assistant in Biology, New York University.
PIERCE, MADELENE E., Graduate Student, Radcliffe College.
POWERS, PHIL B., Instructor in Zoölogy, University of Pennsylvania.
ROBINSON, ELLIS J., Graduate Assistant, New York University.
ROOT, CLINTON W., Assistant in Biochemistry, Princeton University.
SCHECHTER, VICTOR, College of the City of New York.
SCHUETT, J. F., Assistant in Zoölogy, University of Chicago.
SCHWEITZER, MORTON D., Assistant in Zoölogy, Columbia University.
SHAPIRO, HERBERT, Assistant in Zoölogy, Columbia University.
SHAW, C. RUTH, Faculty Member, Kent State College.
SHORE, AGNES, Instructor in Chemistry, Bellevue Medical College, New York University.
SICHEL, FERDINAND J., Assistant Instructor, Washington Square College, New York University.
SOUTHWICK, WALTER E., Student, Harvard University.
SPEICHER, B. R., Graduate Assistant, University of Pittsburgh.
STANCATI, MILTON F., Graduate Assistant, University of Pittsburgh.
STURDIVANT, H. P., Instructor, Columbia University.
TORVIK, MAGNHILD, Graduate Assistant, University of Pittsburgh.
TOWNSEND, GRACE, Fellow, University of Chicago.
TWYEFFORT, LOUIS H., Instructor in Biology, Princeton University.
WALD, GEORGE, Research Assistant, Columbia University.
WALKER, PAUL A., Student, Bowdoin College.
WEINER, ELEANOR, Graduate Student, University of Pennsylvania.
WEISMAN, MAXWELL N., Fellow, College of the City of New York.
WELTY, CARL, Assistant Professor of Biology, Parsons College.
WILDE, MARY H., Graduate Assistant in Botany, New Jersey College for Women.
WILLIAMS, MARY E., 37 Addington Road, Brookline, Massachusetts.
WINSOR, AGNES A., Volunteer Associate in Biology, School of Hygiene, Johns Hopkins University.
WINSOR, CHARLES P., Associate in Biology, School of Hygiene and Public Health, Johns Hopkins University.

Research Assistants

ALDERMAN, EVANGELINE, Graduate Assistant, Wellesley College.
ARMSTRONG, ELEANOR F., Cornell University Medical College.
ASHKENAZ, DAVID M., Assistant in Biology, New York University.
BAILEY, SARAH W., Student, Radcliffe College.
BOYD, MILFORD J., Assistant Graduate Student, University of Cincinnati.
BUTT, CHARLES, Research Assistant, Princeton University.
CARABELLI, A. ALBERT, Medical Student, University of Pennsylvania.
COLDWATER, K. B., Instructor in Zoölogy, University of Missouri.
COLE, ROBERT, 45 King Street, Oberlin, Ohio.
DAVIS, JAMES E., Laskar Fellow and Research Assistant in Medicine, University of Chicago.

DuBois, Anne Marie, Research Assistant, Carnegie Institution of Washington.
Erlanger, Margaret, Harvard University Medical School.
Eyre, Sarah W., Research Assistant, Long Island University.
Fowler, J. R., Research Assistant, University of Chicago.
Francis, Dorothy S., Research Assistant in Biophysics, Memorial Hospital, New York City.
Friedheim, Ernst A. H., Rockefeller Institute.
Graham, Clarence H., National Research Fellow, Johnson Foundation, University of Pennsylvania.
Graubard, Marc A., National Research Council Fellow.
Harnly, Marie L., Assistant in Biology, New York University.
Harryman, Ilene, Lilly Research Laboratories.
Henshaw, Christine T., Assistant Biophysicist, Memorial Hospital, New York City.
Hilsman, Helen, Assistant, University of Pittsburgh.
Holt, Helen, Assistant, Washington Square College, New York University.
Homès, Marcel V. L., Assistant in Botany, University of Brussels.
Larrabee, Martin G., Student, Harvard College.
Parks, Elizabeth K., Instructor in Histology and Embryology, Boston University Medical School.
Parpart, Ethel R., 230 Nassau Street, Princeton, New Jersey.
Rempe, Alois E., Washington University Medical School.
Schluger, Jack, Research Assistant, New York University.
Schmuck, M. Louise, Research Assistant, Carnegie Institution.
Scott, Allan C., Graduate Assistant in Zoölogy, University of Pittsburgh.
Sell, James P., Student, Oberlin College.
Smith, Helen Berenice, Research Assistant, Carnegie Institution of Washington.
Smith, M. Doreen, Research Assistant, University of Toronto.
Smith, Suzanne G., Research Assistant, University of Missouri.
Stokes, Julia C., Research Assistant, Washington University.
Tang, Pei-Sung, Research Fellow, Harvard University.
Tocker, Albert M., Research Assistant, Washington University Medical School.
Tyler, Betty S., California Institute of Technology.
Van Alstyne, Margaret A., Assistant, Harvard University Medical School.
Wade, Lucille W., Lilly Research Laboratories.
Wilson, Hildegard N., Teaching Fellow, New York University and Bellevue Hospital Medical College.
Young, Saul B., Technician, Rockefeller Institute.

Students

BOTANY

Altland, Clair S., American University.
Andrew, Barbara R., Assistant Instructor in Botany, University of Alabama.
Bryan, Hilah F., Smith College.
Hunt, William, Student, Southwestern.
Jackson, John R., Graduate Assistant in Botany, University of Missouri.
Moore, Caroline, Student. University of Pennsylvania.
Perry, Lily M., Graduate Student, Washington University.

EMBRYOLOGY

Alderman, Evangeline, Graduate Assistant, Wellesley College.
Alexander, Lloyd E., Instructor, Fisk University.
Alexanderson, Amelie M., Student, Bryn Mawr College.

BOONE, ELEANOR S., Instructor in Zoölogy, Mills College.
BUCHHEIT, J. ROBERT, Assistant in Zoölogy, University of Illinois.
BUCK, MARGARET A., Student, University of Maine.
CABLE, RAYMOND M., University Fellow, New York University.
CARLSON, J. GORDON, Graduate Student, University of Pennsylvania.
CHASE, HYMAN Y., Graduate Student, Howard University.
CHEN, HSIN T., Student, Harvard University.
COULTER, EDITH A., Student, Goucher College.
DENNY, MARTHA, Radcliffe College.
DERRICKSON, MARY B., Graduate Assistant, Syracuse University.
DICK, GEORGE A., Professor of Animal Industry, University of Pennsylvania.
KASTON, BENJAMIN J., Graduate Student, Yale University.
MAGRUDER, SAMUEL R., Graduate Assistant in Zoölogy, University of Cincinnati.
NEWCOMER, A. VIRGINIA, Radcliffe College.
OPPENHEIMER, JANE M., Student, Bryn Mawr College.
PLYLER, PHYLLIS V., Goucher College.
PRICE, JOHN B., Graduate Student, Stanford University.
SAWYER, ELIZABETH L., 29 Elm Street, Bangor, Maine.
SELL, JAMES P., Oberlin College.
SHEA, MARGARET M., Graduate Assistant, Wellesley College.
SMITH, WILSON F., JR., Student, Cornell University Medical College.
TOWNSEND, GRACE, Fellow, University of Chicago.
WALKER, PAUL A., Bowdoin College.
WEED, MILTON R., Wesleyan University.
WOODRUFF, BETH H., Graduate Assistant, Western Reserve University.
WOODSIDE, GILBERT L., Assistant in Embryology Laboratory, DePauw University.

PHYSIOLOGY

BARNEY, RAYMOND L., Professor of Biology, Middlebury College.
BECK, LYLE V., Graduate Assistant, New York University.
FISHER, KENNETH C., Student, Acadia University.
GAETJENS, LAURA C., Elmira College.
GREEN, DAVID E., Teaching Fellow, New York University.
HEISS, ELIZABETH M., Instructor in Biology, Purdue University.
LUNDSTROM, HELEN M., Graduate Student, University of Pennsylvania.
MCQUESTEN, BARBARA, 14 Still Street, Brookline, Massachusetts.
MICHAELIS, EVA, 1185 Park Avenue, New York City, New York.
MOORE, ELINOR, Student, University of Pennsylvania.
MORGAN, ISABEL M., Student, Stanford University.
NOLL, CLARENCE I., Laboratory Assistant in Chemistry, Trinity College.
PROSSER, C. LADD, Graduate Student and Assistant, Johns Hopkins University.
REID, MARION A., Instructor in Physiology, Boston University School of Medicine.
SCHERP, HENRY W., Assistant, Rockefeller Institute.
SWEETMAN, HARVEY L., Assistant Professor of Entomology, Massachusetts State College.
WILLARD, WILLIAM R., Student, Yale School of Medicine.

PROTOZOÖLOGY

ADELL, JAMES C., Assistant, Teachers College, Columbia University.
AURINGER, JACK, College of City of Detroit.
BROWN, REBECCA R., Columbia University.
BURR, EDITH ROGERS, Columbia University.
CARPENTER, HELENA JANE, Ohio Wesleyan University.
DEE, M. BARBARA, Assistant, Jamaica Plain High School.

ERICSON, ALMA L., Critic Teacher in Biology, Hunter College Model School.
ESKRIDGE, LYDIA C., Technical Assistant, Johns Hopkins University.
FENTON, FRANCES E., Teacher of General Science, Connecticut College.
HENDERSON, LILLIAN O., Instructor in Biology, H. Sophie Newcomb College.
HUTCHINGS, LOIS M., Teacher of High School Biology, Newark, New Jersey.
ICKES, MARGUERITE, Teacher of Biology, Lincoln High School, Cleveland, Ohio.
JAMES, MIRIAM E., Science Teacher in High School, Gloucester, Massachusetts.
ORMSBY, ANDREW A., College of City of Detroit.
SPERRY, HELEN O., Teacher of Biology, Cathedral School of St. Mary.
STEWART, PAUL A., University of Rochester.
WATKINS, EVELYN G., Student, Vassar College.

INVERTEBRATE ZOÖLOGY

AGUAGO, CARLOS S., Assistant Professor of Biology, University of Havana.
ANTHONY, ELIZABETH S., Graduate Student, Brown University.
BACHRACH, JOSEPHINE E., Student, Vassar College.
BAKER, E. G. STANLEY, Student Assistant, DePauw University.
BARRON, DONALD H., Graduate Student, Yale University.
BELCHER, JANE C., Colby College.
BREWSTER, JAMES R., Biological Production, University Film Foundation.
CHASE, HYMAN Y., Graduate Student, Howard University.
CHEN, HSIN T., Student, Harvard University.
CHINN, MARY PRISCILLA, Goucher College.
CLARK, ADELE F., Student, Tufts College.
CLARK, JEAN M., Student, Wilson College.
CLAUSEN, RALPH G., Instructor, Union College.
COHEN, BERNARD M., Assistant in Zoölogy, Johns Hopkins University.
COREY, H. IRENE, Research Assistant and Secretary, University of Pennsylvania.
CROLY, JOHN T., Student, Dartmouth College.
DIMICK, HELEN, Student, Wellesley College.
DREW, R. W., Student, Wesleyan University.
DRUGG, HELEN, Teacher, University of Vermont.
EASTLICK, HERBERT L., Graduate Assistant in Zoölogy, Washington University.
ELLIS, LOLA M., Assistant in Biology Department, Southwestern.
FISH, HAROLD S., Student Assistant, Harvard University.
FORHAN, LAURA JO, Student, University of Montana.
FUCHS, BARRETT, Student Assistant in Biology, American University.
GERSTELL, RICHARD, Student, Dartmouth College.
GLIDDEN, DOROTHY P., Smith College.
GODWIN, MELVIN, Student, DePauw University.
HEGNER, ISABEL, Student, Radcliffe College.
HETRICK, LAWRENCE A., JR., American University.
HOWARD, JOHN W., Student, Hamilton College.
HUSSEY, KATHLEEN L., Fellow in Zoölogy, Ohio Wesleyan University.
JEFFERSON, MARGARET D., Graduate Student, University of Pennsylvania.
JOHNSON, ARLENE C., Student, Wheaton College.
JONES, E. R., Lecturer in Biology, Dalhousie University.
KILGORE, BYRON, JR., Butler University.
KRAMER, THEODORE C., Research Assistant in Biology, Western Reserve University.
LANGSTROTH, MURIEL A., Dalhousie University.
MANN, DONALD R., Investigator, Duke University.
METZNER, JEROME, Fellow, College of the City of New York.
MOMENT, GAIRDNER B., Graduate Student, Yale University.

NICOLL, PAUL A., Graduate Assistant, Washington University.
POMERAT, CHARLES M., Assistant in Biology, Clark University.
RAYE, WILLIAM H., JR., Amherst College.
ROSENBAUM, LOUISE, Laboratory Assistant, University of Pennsylvania.
ROUNTREE, KATHERINE E., Instructor in Biology, Wesleyan College.
SANDERS, ROSALTHA, Student, Yale University.
SMITH, OSGOOD R., Hamilton College.
SOLBERG, ARCHIE N., Instructor in Zoölogy, North Dakota Agricultural College.
STEWART, PAUL A., University of Rochester.
THOMAS, THURLO B., Graduate Assistant, Oberlin College.
WARTERS, MARY, Assistant Professor of Zoölogy, Centenary College.
WESTKAEMPER, SISTER REMBERTA, Head of Department of Biology, College of St. Benedict.
WILLARD, WILLIAM R., Student, Yale School of Medicine.
WISMER, VIRGINIA, Assistant, University of Pennsylvania.
YOUNG, GEORGE D., Student, Acadia University.

3. TABULAR VIEW OF ATTENDANCE

	1927	1928	1929	1930	1931
INVESTIGATORS—Total	294	323	329	337	362
Independent	209	217	234	217	236
Under Instruction	57	81	71	87	83
Research Assistants	28	25	24	33	43
STUDENTS—Total	141	133	125	136	125
Zoölogy	57	57	53	56	55
Protozoölogy	17	16	15	14	17
Embryology	32	29	28	27	29
Physiology	19	15	17	23	17
Botany	16	16	12	16	7
TOTAL ATTENDANCE	435	456	454	473	487
Less Persons registered as both Students and Investigators	1	2	10	14	20
	434	454	444	459	467
INSTITUTIONS REPRESENTED—Total	111	111	123	126	137
By Investigators	89	80	96	95	102
By Students	63	66	64	71	68
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	1	1	–	–	–
By Students	4	1	1	4	4
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	15	13	30	7	8
By Students	8	8	3	2	1

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS, 1931

Acadia University
American University, Washington, D. C.
Amherst College
Barnard College
Bowdoin College
Brown University
Bryn Mawr College
Butler College
C. R. B. Educational Foundation
California Institute of Technology
Carnegie Institution, Cold Spring Harbor
Carnegie Institution of Washington
College of St. Benedict
Columbia University
Cornell University
Cornell University Medical College
Dalhousie University
Dartmouth College
DePauw University
Duke University
Elmira College
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Howard University
Hunter College
Indiana University
Industrial & Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
Johns Hopkins University Medical School
Eli Lilly & Co.
Long Island University
Loyola University
Memorial Hospital of New York City
Morehouse College
Mount Holyoke College
National Research Council
New York State Department of Health
New York University
Oberlin College
Pennsylvania College for Women
Princeton University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
Seton Hill College
Smith College
Sophie Newcomb College
Southwestern
Syracuse University
Tufts College
Union College
United States Department of Agriculture
University of Buffalo
University of Chicago
University of Chicago Medical School
University of Cincinnati
University of Illinois
University of Iowa
University of Minnesota
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Texas
University of Virginia
University of Wisconsin
Vanderbilt University Medical School
Vassar College
Washington University
Washington University Medical School
Wellesley College
Wesleyan University
Western Reserve University
West Virginia University
Wheaton College
Wilson College
Wistar Institute of Anatomy and Biology
Yale University

SCHOLARSHIP TABLES

Lucretia Crocker Scholarships for Teachers in Boston.

Scholarship of $100 supported by a friend of the Laboratory since 1898.

The Edwin S. Linton Memorial Endowment of Washington and Jefferson College, given by Edwin Linton and Margaret Brownson Linton in memory of their son, member of the class of 1913, who gave his life in France during the World War. The endowment amounts to $2,500, and the income therefrom is to be used to encourage study and research at the Marine Biological Laboratory, Woods Hole, Massachusetts.

The Bio Club Scholarship of the College of the City of New York.

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5. EVENING LECTURES, 1931

Tuesday, June 23
DR. LEONOR MICHAELIS ..... "Theory of the Heme Pigments as Oxygen Carriers and as Oxidation Catalysts."

Friday, July 3
DR. E. B. WILSON ..... "The Central Bodies." Illustrated by Original Photomicrographs.

Friday, July 10
DR. G. H. PARKER ..... "Humoral Agents in Nervous Activities with Special Reference to Chromatophores."

Friday, July 17
DR. ELIOT R. CLARK ..... "The Microscopic Study of Cells and Tissues in the Living Mammal."

Friday, July 24
DR. T. H. MORGAN ..... "The Marine Laboratories of the World and Their Work."

Friday, July 31
DR. H. SPEMANN ..... "Experiments on the Amphibian Egg."

Friday, August 7
THE REYNOLD A. SPAETH MEMORIAL LECTURE, delivered by DR. ROSS G. HARRISON ..... "Problems and Methods of Experimental Embryology."

Friday, August 14
DR. F. L. HISAW ..... "The Corpus Luteum and Anterior Lobe Hormones and Their Physical Inter-relationships."

Friday, August 21
DR. CHARLES R. STOCKARD ..... "An Experimental Dog Farm for the Study of Form and Type."

Wednesday, August 26
DR. E. D. CONGDON ..... "Some Impressions, Racial and Cultural, of the Siamese."

Friday, August 28

Dr. J. H. McGregor ..... "Motion Pictures Taken in the Belgian Congo and the Cameroon by the African Expedition (1929–30) of Columbia University and the American Museum of Natural History."

Friday, September 4

Dr. Bradley M. Patton ..... "Micro-moving Pictures Applied to the Study of the Living Embryo."

SPECIAL LECTURES AND MOTION PICTURES

Saturday, July 18

Motion Pictures ..... "Modern Studies of Sulphur."
"Monel Metal."

Thursday, July 23

Dr. A. E. Navez ..... "Geotropism in Plants."

Mr. Leonard Craske ..... "The Art and Uses of Color Photography."

Thursday, August 20

Motion Pictures ..... "Cleaving Eggs of Echinarachnius and Arbacia." (Dr. Henry J. Fry)
"The Redistribution of Granules in Centrifuged Arbacia Eggs." (Dr. E. N. Harvey)
"Arterio-venous Anastomoses." (Dr. E. R. Clark)
"The Function of the Intestinal Villi." (Dr. F. Verzár—comments by Dr. W. E. Garrey)

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6. SHORTER SCIENTIFIC PAPERS, 1931

Tuesday, June 30

Dr. G. S. Dodds ..... "Osteoclasts and Chondroclasts."

Dr. A. W. Pollister ..... "The Architecture of the Liver Cells of Amphiuma."

Dr. G. H. Parker ..... "Passage of Sperms and Eggs through the Mammalian Oviduct."

Tuesday, July 7

Dr. A. C. Redfield ..... "Effect of Hydrogen Ion Concentration and Salt Concentration on the Oxygen Dissociation Constant of Hemocyanin."

Dr. Laurence Irving ..... "The Carbon Dioxide Dissociation Curve of Living Mammalian Muscle."

Dr. E. N. Harvey ..... "Photo-electric Records of Animal Luminescence."

Tuesday, July 14

Dr. H. H. Plough ..... "Some Observations on Self Sterility in Styela."

DR. R. CHAMBERS ..... "Evidence of a Direct Action of the Nucleus on the Cytoplasm in Tissue Cultures."
DR. A. F. HUETTNER ..... "Genetic Continuity of the Central Bodies."

Tuesday, July 21
DR. S. MORGULIS ..... "The Chemistry of Bone Ash."
DR. J. M. JOHLIN ..... "The Enolization of Gelatin by Neutral Salts."
DR. E. S. GUZMAN BARRON ..... "Oxidations Produced by Gonococci."
DR. SHIRO TASHIRO AND
MR. L. H. SCHMIDT ..... "Bile Salts."

Monday, July 27
DR. J. P. TURNER ..... "The Fibrillar System in Euplotes."
DR. DANIEL RAFFEL ..... "Types of Variation Produced by Conjugation in Paramecium aurelia."
DR. RUTH S. LYNCH ..... "Effects of Conjugation in a Number of Clones of Paramecium aurelia."
DR. T. M. SONNEBORN ..... "Crossing Diverse Clones of Paramecium aurelia."

Wednesday, July 29
UNDER THE AUSPICES OF THE SOCIETY OF CELLULAR BIOLOGY
DR. BALDUIN LUCKÉ ..... "The Mechanism of Bacteriotropin Action."
DR. M. H. JACOBS AND
DR. A. K. PARPART ..... "Is the Permeability of the Erythrocyte to Water Decreased by Narcotics?"
DR. L. V. HEILBRUNN ..... "The Action of the Common Cations on the Protoplasmic Viscosity of Amoeba."
DR. R. CHAMBERS ..... "The Formation of Ice Crystals in the Protoplasm of Various Cells."

Tuesday, August 4
DR. E. F. DUBOIS ..... "Surface Temperature and the Radiation of Heat from the Human Body."
DR. PIERRE RIJLANT ..... "Oscillographic Study of the Cardiac Ganglion of Limulus polyphemus."
DR. D. M. WHITAKER ..... "The Change in Rate of Oxygen Consumption at Fertilization of the Eggs of Chaetopterus, Cumingia, Nereis, Arbacia and Fucus."
DR. R. W. GERARD ..... "Phosphocreatin in Nerve in Relation to Activity."

Tuesday, August 11
DR. W. H. F. ADDISON ..... "Aquatic Mammals—A Description of a Special Cell Type in the Cerebellum."

DR. C. C. SPEIDEL ..... "Living Nerve Sprouts."
DR. J. E. KINDRED ..... "Histologic Effects of Ligation on the Vasa of the Spleen of the Albino Rat."
DR. G. S. DERENYI ..... "The Effect of Radium Irradiation upon the Ovaries of the Albino Rat."

Tuesday, August 18

DR. HELEN B. SMITH ..... "Genetic Studies on Selective Segregation of Chromosomes in Sciara."
DR. P. W. WHITING ..... "Local and Correlative Gene Effects in Mosaics of Habrobracon."
DR. C. B. BRIDGES ..... "Specific Modifiers in Drosophila melanogaster."

Tuesday, August 25

DR. PAUL S. HENSHAW ..... "Recovery from X-ray Effects as Observed in Arbacia Eggs."
MR. WARE CATTELL ..... "The Reaction of the Fundulus Ovum to the Direct Electric Current."
DR. E. A. WOLF AND
DR. H. H. COLLINS ..... "The Effect of Ultra-violet Radiation upon the Color Pattern of Triturus."
DR. G. H. PARKER ..... "The Discharge of Nematocysts."
DR. DMITRY N. BORODIN ..... "Biological Spectrum and M-rays." (Motion pictures)

Tuesday, September 1

DR. ALBERT TYLER ..... "Artificial Parthenogenesis in the Eggs of the Pacific Coast Echiuroid, Urechis caupo."
DR. PAUL S. GALTSOFF ..... "Specificity of Sexual Reactions in the Genus Ostrea."
DR. K. B. COLDWATER ..... "The Effect of Sulphydryl Compounds upon Regenerative Growth."
DR. N. A. COBB ..... "The Use of Live Nemas in Zoological Courses in Schools and Colleges."

Thursday, September 3

DR. CHARLOTTE HAYWOOD AND
DR. WALTER S. ROOT ..... "The Cleavage Rate of the Arbacia Egg in the Presence of Carbon Dioxide and Bicarbonate."
DR. ARTHUR K. PARPART AND
DR. M. H. JACOBS ..... "The Action of Acetic Acid and Its Sodium Salt on the Cleavage of Arbacia Eggs."
MR. K. DAN ..... "Cataphoretic Studies of Marine Eggs."
DR. KENNETH COLE ..... "Surface Forces of the Arbacia Egg."
DR. E. N. HARVEY ..... "The Tension at the Surface of Arbacia Eggs, Determined by Centrifugal Force."

Dr. Ethel Browne Harvey ..... "Development of Arbacia Half-Eggs Produced by Centrifugal Force."

Dr. Balduin Lucké ..... "Osmotic Properties of 'Fragments' of Arbacia Eggs Obtained by Centrifugal Force."

Dr. M. H. Jacobs and
Dr. Dorothy R. Stewart ..... "A Method for the Quantitative Measurement of Cell Permeability."

Dr. Dorothy R. Stewart and
Dr. M. H. Jacobs ..... "The Effect of Fertilization on the Permeability of the Arbacia Egg to Ethylene Glycol."

Dr. Miriam Scott Lucas ..... "Recent Observations upon a Type of Fission Undescribed for Ciliates."

Dr. E. C. Cole ..... "Selective Intra-Vitam Staining of Specific Elements in the Integument of the Squid."

Dr. C. C. Speidel ..... "Types of Nerve Regeneration, as Revealed by Prolonged Observation of Individual Fibers in Living Frog Tadpoles."

Dr. H. H. Johnson ..... "Centrioles and Other Cytoplasmic Bodies in Living Cells of Gryllids."

Dr. P. W. Whiting ..... "Genetic Results in Habrobracon Bearing on Maturation and Fertilization."

Mr. L. V. Beck and
Mr. D. E. Green ..... "Oxidation-reduction Potentials of Cytolyzed and Intact Echinoderm Eggs."

Dr. Eric G. Ball ..... "Hemolysis of Fish Erythrocytes by an Impurity in Sodium Chloride."

Dr. G. H. A. Clowes, Dr. I. H. Page and Mr. H. A. Shonle ..... "On the Contrasting Cytolytic Effects Exerted by Soaps of the Type of Sodium Ricinoleate and Sodium Oleate at Different H Ion Concentrations and the Relation of These Effects to the Oil-water Interfacial Tensions Exerted by the Soaps in Question."

Miss Anna Keltch, Miss Ilene Harryman and Dr. G. H. A. Clowes ..... "Influence of H Ion Concentration on the Anesthetic Value of a Series of General and Local Anesthetics and Hypnotics."

Mr. S. A. Corson ..... "The Action of Acid and Alkali on the Protoplasmic Viscosity of Amoeba dubia."

Mr. H. B. Steinbach ..... "The Effect of Salts on the Injury Current of Scallop Muscle."

Dr. Robert Chambers and Mr. D. A. Marsland ..... "The Action of the Common Salts on the Protoplasm of the Echinoderm Egg."

Mr. Morris Belkin ..... "Capping of Oils on Protoplasmic Surfaces."

Demonstrations

Mr. David M. Ashkenaz ..... "The Effect of Sodium and Calcium Chlorides on Changes in Penetrability of Neutral Red."

Dr. Eric G. Ball ..... "Hemolysis of Fish Erythrocytes by an Impurity in Sodium Chloride."

Mr. Morris Belkin ..... "The Capping Phenomenon in Amoeba dubia."

Dr. C. B. Bridges ..... "Apparatus and Designs for Raising Drosophila."

Dr. E. R. Clark, Mrs. E. L. Clark, Dr. H. T. Kirby-Smith and Dr. W. J. Hitschler ..... "Living Tissues as Seen in Transparent Chambers Introduced into the Rabbit's Ear."

Dr. E. C. Cole ..... "Selective Intra-Vitam Staining of Specific Elements in the Integument of the Squid."

Dr. Kenneth Cole ..... "An Egg Crusher."

Dr. E. N. Harvey and Dr. E. B. Harvey ..... "Arbacia Half-cells (Fertilized and Unfertilized) Produced by Centrifugal Force."

Dr. H. H. Johnson ..... "Centrioles and Other Cytoplasmic Bodies in Living Cells of Gryllids."

Dr. Miriam Scott Lucas ..... "Demonstration of Fission of Cyathodidinium piriforme."

Dr. C. W. Metz ..... "Demonstration of Chromosomes of Sciara."

Dr. A. E. Navez ..... "Cardiac Frequency of Anomya as a Function of Temperature."

Dr. Nellie M. Payne ..... "The Effect of Temperature upon the Duration of 'Death Feigning.'"

Mr. F. J. M. Sichel ..... "Apparatus for Studying Tension in Isolated Muscle Cells."

Dr. C. C. Speidel ..... "Nerve Sprouts, Sheath Cells and Myelin Segments in Living Frog Tadpoles."

Dr. Anna R. Whiting, Miss Magnhild M. Torvik, Mrs. Lysbeth H. Benkert and Miss Kathryn A. Gilmore ..... "Exhibit of Mutants and Mosaics in Habrobracon."

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Coolidge, Mr. C. A., Ames Building, Boston, Mass.
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Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
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Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.
McMurrich, Prof. J. P., University of Toronto, Toronto, Canada.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, Calif.
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Baker, Dr. E. H., 5312 Hyde Park Boulevard, Hyde Park Station, Chicago, Ill.

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Box, Miss Cora M., University of Cincinnati, Cincinnati, O.

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Brailey, Miss Miriam E., 800 Broadway, Baltimore, Md.

Bridges, Dr. Calvin B., California Institute of Technology, Pasadena, Calif.

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BUMPUS, PROF. H. C., 76 Carlton Road, Waban, Mass.
BYRNES, DR. ESTHER F., 1803 North Camac Street, Philadelphia, Pa.
CALKINS, PROF. GARY N., Columbia University, New York City, N. Y.
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COLE, DR. ELBERT C., Williams College, Williamstown, Mass.
COLE, DR. LEON J., College of Agriculture, Madison, Wis.
COLLETT, DR. MARY E., Western Reserve University, Cleveland, O.
COLLEY, MRS. MARY W., 36 Argyle Place, Rockville Centre, Long Island, N. Y.
COLTON, PROF. H. S., Box 127, Flagstaff, Ariz.
CONNOLLY, DR. C. J., Catholic University, Washington, D. C.

COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Me.
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CRAMPTON, PROF. H. E., Barnard College, Columbia University, New York City, N. Y.
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CURTIS, DR. MAYNIE R., Crocker Laboratory, Columbia University, New York City, N. Y.
CURTIS, PROF. W. C., University of Missouri, Columbia, Mo.
DAVIS, DR. ALICE R., Castle Point, Hoboken, N. J.
DAVIS, DR. DONALD W., College of William and Mary, Williamsburg, Va.
DAWSON, DR. A. B., Harvard University, Cambridge, Mass.
DAWSON, DR. J. A., College of the City of New York, New York City, N. Y.
DEDERER, DR. PAULINE H., Connecticut College, New London, Conn.
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DONALDSON, PROF. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
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GAGE, PROF. S. H., Cornell University, Ithaca, N. Y.

GARREY, PROF. W. E., Vanderbilt University Medical School, Nashville, Tenn.
GATES, DR. F. L., 31 Fayerweather Street, Cambridge, Mass.
GATES, PROF. R. RUGGLES, University of London, London, England.
GEISER, DR. S. W., Southern Methodist University, Dallas, Tex.
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GOLDFORB, PROF. A. J., College of the City of New York, New York City, N. Y.
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GRAY, PROF. IRVING E., Duke University, Durham, N. C.
GREENMAN, PROF. M. J., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
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HARVEY, PROF. E. N., Princeton University, Princeton, N. J.
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HEILBRUNN, DR. L. V., University of Pennsylvania, Philadelphia, Pa.
HESS, PROF. WALTER N., Hamilton College, Clinton, N. Y.
HINRICHS, DR. MARIE A., 1824 Blue Island Avenue, Chicago, Ill.
HISAW, DR. F. L., University of Wisconsin, Madison, Wis.

HOADLEY, DR. LEIGH, Harvard University, Cambridge, Mass.
HOGUE, DR. MARY J., 503 N. High Street, West Chester, Pa.
HOLMES, PROF. S. J., University of California, Berkeley, Calif.
HOOKER, PROF. DAVENPORT, University of Pittsburgh, Pittsburgh, Pa.
HOPKINS, DR. HOYT S., New York University, College of Dentistry, New York City, N. Y.
HOWARD, DR. HARVEY J., Washington University, St. Louis, Mo.
HOWE, DR. H. E., 2702 36th Street, N.W., Washington, D. C.
HOYT, DR. WILLIAM D., Washington and Lee University, Lexington, Va.
HUMPHREY, MR. R. R., University of Buffalo, School of Medicine, Buffalo, N. Y.
HYMAN, DR. LIBBIE H., University of Chicago, Chicago, Ill.
INMAN, PROF. ONDESS L., Antioch College, Yellow Springs, O.
IRVING, PROF. LAURENCE, University of Toronto, Toronto, Canada.
IRWIN, DR. MARIAN, Rockefeller Institute, New York City, N. Y.
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JENNINGS, PROF. H. S., Johns Hopkins University, Baltimore, Md.
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JOHNSON, PROF. GEORGE E., State Agricultural College, Manhattan, Kans.
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KEEFE, REV. ANSELM M., St. Norbert College, West Depere, Wis.
KENNEDY, DR. HARRIS, Readville, Mass.
KINDRED, DR. J. E., University of Virginia, Charlottesville, Va.
KING, DR. HELEN D., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
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KNOWLTON, PROF. F. P., Syracuse University, Syracuse, N. Y.
KOSTIR, DR. W. J., Ohio State University, Columbus, O.
KRIBS, DR. HERBERT, 202A Copley Road, Upper Darby, Pa.
KUYK, DR. MARGARET P., Westbrook Ave., Richmond, Va.
LANCEFIELD, DR. D. E., Columbia University, New York City, N. Y.
LANGE, DR. MATHILDE M., Wheaton College, Norton, Mass.

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LILLIE, PROF. FRANK R., University of Chicago, Chicago, Ill.
LILLIE, PROF. RALPH S., University of Chicago, Chicago, Ill.
LINTON, PROF. EDWIN, University of Pennsylvania, Philadelphia, Pa.
LOEB, PROF. LEO, Washington University Medical School, St. Louis, Mo.
LOEB, MRS. LEO, 812 Boland Place, St. Louis, Mo.
LOWTHER, MRS. FLORENCE DEL., Barnard College, Columbia University, New York City, N. Y.
LUCKÉ, PROF. BALDUIN, University of Pennsylvania, Philadelphia, Pa.
LUND, DR. E. J., University of Texas, Austin, Tex.
LUSCOMBE, MR. W. O., Woods Hole, Mass.
LYNCH, DR. CLARA J., Rockefeller Institute, New York City, N. Y.
LYNCH, DR. RUTH STOCKING, Johns Hopkins University, Baltimore, Md.
LYON, PROF. E. P., University of Minnesota, Minneapolis, Minn.
MACDOUGALL, DR. MARY S., Agnes Scott College, Decatur, Ga.
MCCLUNG, PROF. C. E., University of Pennsylvania, Philadelphia, Pa.
MCGEE, DR. ANITA NEWCOMB, Box 363, Southern Pines, N. C.
MCGREGOR, DR. J. H., Columbia University, New York City, N. Y.
MCNAIR, DR. G. T., 1624 Alabama Street, Lawrence, Kans.
MACKLIN, DR. CHARLES C., School of Medicine, University of Western Ontario, London, Canada.
MALONE, PROF. E. F., University of Cincinnati, Cincinnati, O.
MANWELL, DR. REGINALD D., Syracuse University, Syracuse, N. Y.
MARTIN, PROF. E. A., College of the City of New York, New York City, N. Y.
MAST, PROF. S. O., Johns Hopkins University, Baltimore, Md.
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MEDES, DR. GRACE, University of Minnesota, Minneapolis, Minn.
MEIGS, DR. E. B., Dairy Division Experiment Station, Beltsville, Md.
MEIGS, MRS. E. B., 1736 M Street, N.W., Washington, D. C.
METCALF, PROF. M. M., Johns Hopkins University, Baltimore, Md.
METZ, PROF. CHARLES W., Johns Hopkins University, Baltimore, Md.
MICHAELIS, DR. LEONOR, Rockefeller Institute, New York City, N. Y.
MILLER, DR. HELEN M., Yale University, New Haven, Conn.
MINER, DR. ROY W., American Museum of Natural History, New York City, N. Y.

MITCHELL, DR. PHILIP H., Brown University, Providence, R. I.
MOORE, DR. CARL R., University of Chicago, Chicago, Ill.
MOORE, PROF. GEORGE T., Missouri Botanical Garden, St. Louis, Mo.
MOORE, PROF. J. PERCY, University of Pennsylvania, Philadelphia, Pa.
MORGULIS, DR. SERGIUS, University of Nebraska, Lincoln, Nebr.
MORRILL, PROF. A. D., Hamilton College, Clinton, N. Y.
MORRILL, PROF. C. V., Cornell University Medical College, New York City, N. Y.
MULLER, DR. H. J., University of Texas, Austin, Tex.
NABOURS, DR. R. K., Kansas State Agricultural College, Manhattan, Kans.
NEAL, PROF. H. V., Tufts College, Tufts College, Mass.
NEWMAN, PROF. H. H., University of Chicago, Chicago, Ill.
NICHOLS, DR. M. LOUISE, Dreycott Apartments, Haverford, Pa.
NOBLE, DR. GLADWYN K., American Museum of Natural History, New York City, N. Y.
NONIDEZ, DR. JOSÉ F., Cornell University Medical College, New York City, N. Y.
OKKELBERG, DR. PETER, University of Michigan, Ann Arbor, Mich.
OSBURN, PROF. R. C., Ohio State University, Columbus, O.
OSTERHOUT, PROF. W. J. V., Rockefeller Institute, New York City, N. Y.
PACKARD, DR. CHARLES, Columbia University, Institute of Cancer Research, 1145 Amsterdam Ave., New York City, N. Y.
PAGE, DR. IRVINE H., Rockefeller Institute, New York City, N. Y.
PAPANICOLAOU, DR. GEORGE N., Cornell University Medical College, New York City, N. Y.
PAPPENHEIMER, DR. A. M., Columbia University, New York City, N. Y.
PARKER, PROF. G. H., Harvard University, Cambridge, Mass.
PATON, PROF. STEWART, Princeton University, Princeton, N. J.
PATTEN, DR. BRADLEY M., Western Reserve University, Cleveland, O.
PATTEN, PROF. WILLIAM, Dartmouth College, Hanover, N. H.
PAYNE, PROF. F., University of Indiana, Bloomington, Ind.
PEARL, PROF. RAYMOND, Institute for Biological Research, 1901 East Madison Street, Baltimore, Md.
PEARSE, PROF. A. S., Duke University, Durham, N. C.
PEEBLES, PROF. FLORENCE, California Christian College, Los Angeles, Calif.
PHILLIPS, DR. E. F., Cornell University, Ithaca, N. Y.
PHILLIPS, DR. RUTH L., Western College, Oxford, O.
PIKE, PROF. FRANK H., 437 West 59th Street, New York City, N. Y.

PINNEY, DR. MARY E., Milwaukee-Downer College, Milwaukee, Wis.
PLOUGH, PROF. HAROLD H., Amherst College, Amherst, Mass.
POLLISTER, DR. A. W., Columbia University, New York City, N. Y.
POND, DR. SAMUEL E., University of Pennsylvania, School of Medicine, Philadelphia, Pa.
PRATT, DR. FREDERICK H., Boston University, School of Medicine, Boston, Mass.
RAFFEL, DR. DANIEL, Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.
RAND, DR. HERBERT W., Harvard University, Cambridge, Mass.
RANKIN, PROF. W. M., Princeton University, Princeton, N. J.
REDFIELD, DR. ALFRED C., Harvard University, Cambridge, Mass.
REESE, PROF. ALBERT M., West Virginia University, Morgantown, W. Va.
REINKE, DR. E. E., Vanderbilt University, Nashville, Tenn.
REZNIKOFF, DR. PAUL, Cornell University Medical College, New York City, N. Y.
RHODES, PROF. ROBERT C., Emory University, Atlanta, Ga.
RICE, PROF. EDWARD L., Ohio Wesleyan University, Delaware, O.
RICHARDS, PROF. A., University of Oklahoma, Norman, Okla.
RICHARDS, DR. O. W., Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.
RIGGS, MR. LAWRASON, JR., 25 Broad Street, New York City, N. Y.
ROBERTSON, PROF. W. R. B., 1803 Anderson Street, Manhattan, Kans.
ROGERS, PROF. CHARLES G., Oberlin College, Oberlin, O.
ROMER, DR. ALFRED S., University of Chicago, Chicago, Ill.
ROOT, DR. W. S., Syracuse Medical School, Syracuse, N. Y.
SANDS, MISS ADELAIDE G., 562 King Street, Port Chester, N. Y.
SCHRADER, DR. FRANZ, Department of Zoölogy, Columbia University, New York City, N. Y.
SCHRAMM, PROF. J. R., University of Pennsylvania, Philadelphia, Pa.
SCOTT, DR. ERNEST L., Columbia University, New York City, N. Y.
SCOTT, PROF. G. G., College of the City of New York, New York City, N. Y.
SCOTT, PROF. JOHN W., University of Wyoming, Laramie, Wyo.
SCOTT, PROF. WILLIAM B., 7 Cleveland Lane, Princeton, N. J.
SHULL, PROF. A. FRANKLIN, University of Michigan, Ann Arbor, Mich.
SHUMWAY, DR. WALDO, University of Illinois, Urbana, Ill.
SIVICKIS, DR. P. B., Pasto deze 130, Kaunas, Lithuania.
SNOW, DR. LAETITIA M., Wellesley College, Wellesley, Mass.
SNYDER, PROF. CHARLES D., Johns Hopkins University Medical School, Baltimore, Md.

SOLLMAN, DR. TORALD, Western Reserve University, Cleveland, O.
SONNEBORN, DR. T. M., Johns Hopkins University, Baltimore, Md.
SPEIDEL, DR. CARL C., University of Virginia, University, Va.
SPENCER, PROF. H. J., 24 West 10th Street, New York City, N. Y.
STARK, DR. MARY B., New York Homeopathic Medical College and Flower Hospital, New York City, N. Y.
STEWART, DR. DOROTHY R., Skidmore College, Saratoga Springs, N. Y.
STOCKARD, PROF. C. R., Cornell University Medical College, New York City, N. Y.
STOKEY, DR. ALMA G., Mount Holyoke College, South Hadley, Mass.
STRONG, PROF. O. S., College of Physicians and Surgeons, 630 West 168th Street, New York City, N. Y.
STUNKARD, DR. HORACE W., New York University, University Heights, N. Y.
STURTEVANT, DR. ALFRED H., California Institute of Technology, Pasadena, Calif.
SUMWALT, DR. MARGARET, University of Pennsylvania, Medical School, Philadelphia, Pa.
SWETT, DR. FRANCIS H., Duke University Medical School, Durham, N. C.
TASHIRO, DR. SHIRO, Medical College, University of Cincinnati, Cincinnati, O.
TAYLOR, WILLIAM R., University of Michigan, Ann Arbor, Mich.
TENNENT, PROF. D. H., Bryn Mawr College, Bryn Mawr, Pa.
THATCHER, MR. LLOYD E., Spring Hill, Tenn.
TRACY, PROF. HENRY C., University of Kansas, Lawrence, Kans.
TREADWELL, PROF. A. L., Vassar College, Poughkeepsie, N. Y.
TURNER, PROF. C. L., Northwestern University, Evanston, Ill.
TYLER, DR. ALBERT, California Institute of Technology, Pasadena, Calif.
UHLEMEYER, MISS BERTHA, Washington University, St. Louis, Mo.
UHLENHUTH, DR. EDUARD, University of Maryland, School of Medicine, Baltimore, Md.
UNGER, DR. W. BYERS, Dartmouth College, Hanover, N. H.
VAN DER HEYDE, DR. H. C., Galeria, Corse, France.
VISSCHER, DR. J. PAUL, Western Reserve University, Cleveland, O.
WAITE, PROF. F. C., Western Reserve University Medical School, Cleveland, O.
WALLACE, DR. LOUISE B., Spelman College, Atlanta, Ga.
WARD, PROF. HENRY B., University of Illinois, Urbana, Ill.
WARREN, DR. HERBERT S., Department of Biology, Temple University, Philadelphia, Pa.
WARREN, PROF. HOWARD C., Princeton University, Princeton, N. J.

WENRICH, DR. D. H., University of Pennsylvania, Philadelphia, Pa.
WHEDON, DR. A. D., North Dakota Agricultural College, Fargo, N. D.
WHEELER, PROF. W. M., Museum of Comparative Zoölogy, Cambridge, Mass.
WHERRY, DR. W. B., Cincinnati Hospital, Cincinnati, O.
WHITAKER, DR. DOUGLAS M., Stanford University, Stanford University, Calif.
WHITE, DR. E. GRACE, Wilson College, Chambersburg, Pa.
WHITING, DR. PHINEAS W., University of Pittsburgh, Pittsburgh, Pa.
WHITNEY, DR. DAVID D., University of Nebraska, Lincoln, Nebr.
WIEMAN, PROF. H. L., University of Cincinnati, Cincinnati, O.
WILLIER, DR. B. H., University of Chicago, Chicago, Ill.
WILSON, PROF. H. V., University of North Carolina, Chapel Hill, N. C.
WILSON, DR. J. W., Brown University, Providence, R. I.
WITSCHI, PROF. EMIL, University of Iowa, Iowa City, Ia.
WOGLOM, PROF. WILLIAM H., Columbia University, New York City, N. Y.
WOODRUFF, PROF. L. L., Yale University, New Haven, Conn.
WOODWARD, DR. ALVALYN E., Zoölogy Department, University of Michigan, Ann Arbor, Mich.
YOUNG, DR. B. P., Cornell University, Ithaca, N. Y.
YOUNG, DR. D. B., University of Maine, Orono, Me.
ZELENY, DR. CHARLES, University of Illinois, Urbana, Ill.

THE REACTION OF THE ERYTHROCYTES OF VERTEBRATES, ESPECIALLY FISHES, TO VITAL DYES

ALDEN B. DAWSON

(From the Zoölogical Laboratories, Harvard University, and the Marine Biological Laboratory, Woods Hole, Massachusetts)

In the erythrocytes of most vertebrates, but especially of fishes and amphibians, discrete granules are characteristically present. In the urodele, *Necturus*, bipolar clusters of such granules are regularly found in mature red blood cells. They are visible in fresh preparations, demonstrable as basophilic bodies with Wright or Giemsa staining, are blackened with osmic acid and silver salts, and are stained with iron hæmatoxylin after Helly fixation. Accordingly, there is no doubt that they are preëxistent structures and are not induced in supravital preparations by the action of the dyes.

However, secondary granules may also appear in such cells; the concentration of the dye, age of the preparation, brilliancy of illumination, and increase in temperature being effective as formative factors, influencing the rate and manner of their appearance (Dawson, 1928, 1929, 1930). Moreover, with higher concentrations of dye, the red cells may also exhibit elaborate patterns of reticulation. The genetic relation of both types of granules, preëxisting and induced, to the reticulated substance is an unsettled question. Morphologically there is no distinction between the two types and both are frequently enclosed in the reticular filaments. The reticulation pattern is apparently derived, through a reaction with the vital dye, from the basophilic substance which occurs diffusely in erythrocytes, and secondary granules are regarded by some as the same substance in a different form.

The literature on this subject is voluminous and many different views are advanced regarding the significance of the protoplasmic constituents reacting with vital dyes. First of all, the ability to react with a given vital dye may not be a specific characteristic of a single substance within the cytoplasm. Again, in many instances, the materials reacting with vital dyes are not always readily demonstrated by other technical methods. *Necturus* erythrocytes appear to be exceptionally favorable in this respect. Moreover, the amount of material reacting with vital dyes appears to decrease gradually as the erythrocytes differentiate, but the acquisition of hemoglobin in a given species proceeds only up to a certain stage at which the cell is said to be mature. Since the erythrocytes in different vertebrates do not attain at maturity the same relative degree

of differentiation, the picture in supravital preparations is subject to great variation; the individual changes which occur in the maturation of the red blood cells may apparently proceed at different rates and to different degrees in different species. Accordingly, the relative concentration of hemoglobin within the erythrocytes of different species appears to be to some extent independent of the amount and distribution of material reacting with vital dyes. These are only some of the factors which complicate the picture and make generalizations almost impossible. Coupled with these there is also the lack of complete information about the stages of maturation of these cells in many species. In other cases, too, findings by one method have not been adequately checked and confirmed by other technics.

Various theories of the origin and nature of the vitally-stained bodies have been advanced. Several earlier workers suggested a nuclear or nucleolar origin (Giglio-Tos, 1896; Jolly, 1903; Sabrazès et Muratet, 1908), while others have apparently confused them with centrosomes (von Apáthy, 1897; Bremer, 1895; Dehler, 1895; Eisen, 1897–1899; Golgi, 1920) when demonstrated by non-vital staining methods such as silver nitrate impregnation or staining with iron hæmatoxylin. They were also identified as intracellular parasites, but this view was soon dropped (Sabrazès et Muratet, 1900).

At present the vital granules are generally regarded as of cytoplasmic origin. Giglio-Tos (1896) suggested that they represented hemoglobin-forming substances and Yoffey (1929) is inclined to favor this view. Several modern investigators (Ferrari, 1930; Villa, 1930; Knoll, 1931) have advanced evidence in favor of an intranuclear origin of hemoglobin, and this interpretation accordingly would support the old hypothesis of Giglio-Tos that vital granules arise as nuclear emissions and are concerned in the elaboration of hemoglobin. On the basis of the reaction of these bodies with silver salts and osmic acid, they have been regarded as possibly homologous with the Golgi apparatus—dictyosomes (Bhattacharya and Brambell, 1925; Dawson, 1928, 1930; Dornesco and Steopoe, 1930*a*, 1930*b*; Urtubey, 1927). Nittis (1930) described a surface granule which in his opinion might be interpreted as the point of separation of two daughter cells or as a part of a trophospongial system. Nittis' view seems untenable, as the single granule is intracellular and may frequently exhibit Brownian motion (Dawson, 1931). Many tend to class all vital granules as artifacts (Chlopin, 1927; Weidenreich, 1903), failing to distinguish sharply between preëxistent and induced bodies (Beams, 1930).

The number and distribution of vital granules in the erythrocytes of vertebrates vary during the differentiation of the cell. Usually they are

not present in very young cells and are reduced or even disappear in old cells. In the intermediate stages they are frequently numerous and conspicuous. In the mature cells of different vertebrates in which they still persist, vital granules frequently occur in rather definite numbers and have a characteristic position within the cell. The more common types of distribution are single unipolar; multiple, clustered unipolar; multiple, clustered bipolar; multiple perinuclear; and multiple scattered or diffuse (Dawson and Charipper, 1929; Dawson, 1930, 1931).

The number and distribution of vital granules in the erythrocytes of cyclostomes and fishes vary considerably. They were apparently recognized as early as 1896 by Giglio-Tos, who observed them in the blood cells of the lamprey and called attention to the striking Brownian movements they exhibited. A few years later Sabrazès and Muratet (1900) described "corpuscles mobiles" within the erythrocytes of *Hippocampus*. They were irregularly distributed, with usually five to ten granules within a cell.

In the same year these investigators extended their observations to several more fishes. In *Torpedo oculata* the granules were numerous and distributed diffusely around the nucleus. The cells usually contained as many as forty granules although some had as few as three, four, and five. All granules exhibited Brownian movement. They were unequal in size; some were coupled and some elongated or compressed. In *Raia pastinaca* the bodies were frequently oval and relatively large but were not so numerous as in *Torpedo*. The blood cells of *Syngnathus typhle* apparently did not contain any granules. In *Petromizon marinus* and *Alosa finta* the granules were present but not numerous. In adult *Anguilla vulgaris* they were absent. In 1902, however, Sabrazès and Muratet found opportunity to examine the blood of some young eels, 6 to 7 cm. long. In these, vital granules with limited Brownian movement were present in about twenty per cent of the erythrocytes. They varied greatly in size, with usually one to three present in any one cell. In *Torpedo marmorata* (Sabrazès et Muratet, 1908) the number and distribution of vital granules is very similar to *Torpedo oculata*.

Jokl (1925) made an intensive study of the erythrocytes of *Raia clavata* and *Raia batis* and found in the main that the red blood cells were very like those of *Torpedo* in the number, size, and distribution of the vital granules. Further observations on the elasmobranchs were made by Lewis and Lewis (1926), who described numerous diffusely scattered perinuclear bodies in the dogfish and skate (*Raia erinacea*). These workers also described one to three neutral red granules in the erythrocytes of the sculpin, but illustrated the erythrocytes of the hake

and cunner as lacking any granular inclusions. Stolz (1928) found that the erythrocytes of *Cyprinus carpio* contained numerous granules diffusely distributed about the nucleus. Yoffey (1929) confirmed the observations of Jokl (1925) on the red blood cells of *Raia clavata* and *Raia batis,* and noted numerous fine basophilic granules in the mature cells of *Trigla gurnardus.* Dornesco and Steopoe (1930*a*) found that the erythrocytes of the dogfish are typical of the elasmobranchs in possessing numerous, scattered, actively motile granules. They (1930*b*) also investigated the blood of several marine teleosts, *Syngnathus acus, Blennius pholis, Solea vulgaris, Pleuronectes platessa, Gobius paganellus, Cottus bubalis, Labrus melops, Onos mustella* and *Nerophis lumbriciformis.* In all of these they found that the erythrocytes contained uniformly one granule, usually located eccentrically near one pole of the nucleus. The conditions in the erythrocytes of *Ameiurus nebulosus* are the same as in the marine teleosts (Dawson, 1931).

In the erythrocytes of the fishes studied the distribution of vital granules is not so variable as in the Amphibia, being limited chiefly to two types, single unipolar and multiple perinuclear, restricted or diffuse. The characteristic unipolar and bipolar clusters appearing in the red blood cells of many urodeles are not found. It has seemed advisable to extend the observations on the blood of fishes, with a view to obtaining more information regarding the relationships between the preëxistent and induced granules and the reticular substance as revealed by vital dyes.

Material and Methods

During the summer of 1931 the blood of seventeen different species of fish, taken in the vicinity of Woods Hole, was examined. Supravital staining was carried out by the dry dye-film method, using neutral red alone, neutral red in combination with Janus green, and brilliant cresyl blue. The concentration of neutral red, 1:1250, previously used on amphibian blood proved satisfactory in the case of fishes, but more Janus green had to be added to obtain a clear view of the chondriosomes. Films of brilliant cresyl blue were made with a saturated solution in absolute alcohol. Permanent preparations of supravitally stained blood were made by the method of Scott (1928). Smears stained by Wright's method were also prepared. In all cases the blood was freshly drawn from the heart of living fish.

It is essential that neutral red alone be used to check neutral red-Janus green B preparations, since the toxicity of Janus green may cause injuries which result in more rapid induction of bodies stainable with neutral red. On the other hand, Janus green B cannot safely be used alone, since bodies regularly stained with neutral red may take up Janus

green and confuse the chondriosome picture. However, when the dyes are used in combination, the neutral red displays a greater affinity for the bodies which are regularly stained by it and the Janus green reaction is accordingly confined entirely to the chondriosomes. The brilliant cresyl blue was applied at such high concentrations that the nuclei were stained and the reticular patterns produced almost immediately, and there did not appear to be any progressive induction of formed bodies (granules) while the preparations were being studied for reticulation patterns.

Description

Before proceeding to the description of the reactions of the erythrocytes of the different fishes to vital dyes, certain general features of these reactions will be discussed. In most fishes there are sufficient immature red cells, in varying stages of differentiation, that the pictures presented after supravital staining lack uniformity. This makes the problem of interpretation more difficult, since the differences in hemoglobin concentration in some cases are scarcely perceptible. Differences in the degree of maturity of such cells are most strikingly demonstrated with brilliant cresyl blue and the reticulation patterns furnish an excellent index of cell age. The presence of young red cells in the blood is readily confirmed in smears differentially stained by Wright's method, where varying degrees of basophilia and polychromasia are clearly demonstrated.

As the erythrocytes mature the amount of material reacting with brilliant cresyl blue gradually decreases and the patterns of reticulation also undergo changes in form and distribution. In very young cells the entire cytoplasm is filled with fine, densely massed granules with little evidence of real reticulation. In succeeding stages of differentiation the pattern assumes a true reticular form. At first the meshes are small and distributed throughout the whole cell. Later the meshes are more open and the reticulation does not extend completely to the periphery of the cell, assuming the form of a perinuclear wreath. As the cells grow older, the meshes are still wider and the filaments eventually are partially interrupted, forming an open, fragmented wreath. As the reticular substance is further reduced, the filaments of the net are more frequently interrupted and the wreath-like form as well as the reticular appearance is lost. The more or less separated filaments then appear radially arranged about the nucleus, with very fine fragments interspersed between them. With the further disappearance of reticular substance the pattern is reduced to scattered, irregular, short filaments and fine, dust-like granules. In final stages the filamentous form may practically disappear, leaving a variable number of fine granules.

It is difficult to determine whether the reticular substance, which is demonstrated with concentrations of the vital dye sufficiently high to stain the nuclei immediately, is of the same constitution as the relatively large granules which may be induced in similar blood cells by a prolonged exposure to the same dye in a lesser concentration, which will not stain the nucleus or produce reticulation. At present, it seems impossible to decide this question. On the other hand, it is frequently possible to distinguish clearly between preëxistent vital granules and the induction patterns, since in many cases the former may be seen in fresh untreated cells or may be demonstrated in fixed cells by a variety of reliable technical methods.

With high concentrations of brilliant cresyl blue, nuclei, nucleoli (when present), preëxistent and induced granules, and reticular substance are all stained. The nuclei are pale blue, nucleoli deep blue, all granules deep blue-purple and the reticular substance a red-violet. The nucleoli, varying in number from one to three, are large and conspicuous in the erythroblasts but become progressively smaller as the cells mature. They may be distinguished as minute bodies in cells of the "wreath" stage but seldom persist in more mature forms. The difference in the color reaction of the granules and the reticular substance may or may not be significant. The granules are sharply limited, dense, and highly refractile, while the reticular substance has an irregular appearance as if formed by the aggregation of very fine particles. The difference in physical state accordingly may explain the different shades presented by the two types of bodies.

The form and distribution of chondriosomes within the erythrocytes of the fishes vary in the different stages of maturity, but are nevertheless quite characteristic for the type of cell. In the younger stages they are quite numerous, granular, and diffusely scattered throughout the cytoplasm. In later stages the number is greatly reduced and they are more closely aggregated about the nucleus. Their form, too, may be changed, many appearing as long, tortuous bodies. In fully mature cells the chondriosomes are usually entirely of the filamentous type, although occasional granular forms are seen. Some of these are not true granules but represent filaments oriented lengthwise between the two flattened surfaces of the erythrocyte and may be observed to shift in position as the cell is modified by the injurious effects of the Janus green.

It is difficult to obtain sharp images of chondriosomes in the fresh cell, and relatively high concentrations of dye must be used. As the staining progresses the chondriosomes are first seen as hazy blue-green bodies, and at this time the nuclear outline is also indistinct. Shortly both the nuclear membrane and chondriosomes are distinctly seen and

the cytoplasm of the cell appears clearer. While these changes are taking place striking movements of the mitochondria may be observed. Filaments which were closely applied to the nucleus may frequently swing out to lie at right angles to the nuclear surface or may move completely away, and others which were seen on end may assume a position parallel with the flattened surface of the cell.

For purposes of description the fishes studied at Woods Hole will be divided into two major groups, based on the distribution of the primary or preëxistent granules as demonstrated by low concentrations of neutral red. In the first group the vitally stained granules are usually single, with occasionally one or more accessory bodies, and are located eccentrically near one pole of the nucleus. In the second group the granules are numerous and may either have a definite perinuclear arrangement or be scattered more or less diffusely throughout the cytoplasm. The reaction of the primary granules to Wright's stain might also be used as a basis of classification, since in some fishes the granules uniformly give a basophilic reaction; in others this reaction is limited to a varying number of cells; while in still others the granules always remain unstained. The three second groups would form natural subdivisions of the major Group I, which is based primarily on the distribution of granules, but in Group II this would not hold true since in no instances do the perinuclear or diffusely arranged granules react uniformly with Wright's stain.

Group I

Toadfish, *Opsanus tau* (Linnæus). With neutral red the primary granules ordinarily appear as single, unipolar bodies, although one or more accessory granules are often encountered (Fig. 1, *b* and *c*). The accessory granules are usually small, but in some cases all the granules are of equal size, resembling the variations described for *Ameiurus* (Dawson, 1931). After long exposure to neutral red the granules are increased in number and tend to form clusters about the primary bodies. Later, other secondary granules appear irregularly throughout the cell.

The mitochondria are relatively few, varying in number from three to six. They usually appear as wavy filaments but are frequently dilated to encapsulate a large spherical refractile body. These bodies are readily seen after the Janus green has been reduced. They do not take up neutral red and their significance is not known.

Very little reticular substance is demonstrated by brilliant cresyl blue. The filaments are short and are usually radially arranged about the nucleus. Some end in contact with the cell membrane. Primary granules and a variable number of secondary granules are stained with

this dye. In the cells with the least reticulation practically no induction of granules has taken place (Fig. 1, *d*). The cells with the most reticulation contain many more granules (Fig. 1, *a*). All the cells appear relatively mature. An occasional cell in the "wreath" stage with a clear border was encountered, but there were no younger stages.

In smears stained by Wright's method the primary granules appear as distinct basophilic bodies. No basophilic erythrocytes are present and only a few cells exhibit polychromasia.

Tautog, *Tautoga onitis* (Linnæus). The neutral red patterns are quite similar to those of the toadfish (Fig. 4, *a* and *b*). A number of erythrocytes possess granular mitochondria, but in the majority of the cells they are filamentous. The reticular substance is scanty in most cells (Fig. 4, *d*), but a few "wreath" stages were seen (Fig. 4, *c*). Occasional small cells (erythroblasts) with a dense reticulation and a conspicuous nucleolus are present.

With Wright's stain the primary granules are stained blue and a few erythrocytes show basophilia and polychromasia. The number of immature red cells is, however, very small.

Cunner, *Tautogolabrus adspersus* (Walbaum). The erythrocytes of this fish very closely resemble in their staining reactions those of the two preceding forms (Fig. 6, *a* and *b*). Fewer immature cells were noted than in the tautog, and in general the reticulation patterns are very meager (Fig. 6, *c* and *d*). The primary granules also appear as basophilic bodies with Wright's stain.

Sea Bass, *Centropristes striatus* (Linnæus). The primary neutral red bodies are the same as in the erythrocytes of the preceding fishes (Fig. 3, *a*). Induction of secondary granules occurs rather freely (Fig. 3, *b*), but even when the secondary staining effect has appeared there are a number of apparently mature cells which do not show any reaction to the dye. The mitochondria are relatively few, the filamentous form predominating.

With brilliant cresyl blue many cells fail to show any reticular patterns but contain only granules such as are seen after moderate induction with neutral red. Other cells contain a variable number of reticular filaments (Fig. 3, *c*), but the "wreath" stage was observed in only a few cells (Fig. 3, *d*). Occasional young cells with a fine, dense reticulum and conspicuous nucleoli were encountered.

In the sea bass virtually all the erythrocytes stain orthochromatically with the eosin in Wright's stain. Polychromasia is rarely seen and occasional round basophilic erythroblasts are present. The primary granules demonstrated so readily with neutral red are not usually stained in the smears. Some of the larger granules appear as distinct baso-

philic bodies, other smaller granules are barely distinguishable, and many cells appear not to have any granular inclusions.

Sea Robin, *Prionotus carolinus* (Linnæus). The erythrocytes of the sea robin in their staining reactions are very like those of the sea bass (Fig. 2). The mitochondria, however, usually appear as short rods and granules rather than filaments. The reticular patterns are very sparse, being represented by scattered particles radially aligned about the nucleus (Fig. 2, *d*). All the cells appear to be mature. This conclusion is supported by the staining reactions of the erythrocytes with Wright's stain. No granular inclusions could be distinguished in the stained smears.

Scup, *Stenotomus chrysops* (Linnæus). The neutral red patterns and mitochondria present no unusual features (Fig. 7, *a* and *c*). The reticular patterns are quite variable, although in the majority of cells the filaments are reduced to a minimum (Fig. 7, *b*). Young cells with a dense reticulum and conspicuous nucleoli are frequently seen and stages with fragmented open-meshed "wreaths" are quite numerous (Fig. 7, *d*). The presence of such immature cells is confirmed by an examination of stained smears, but the preëxistent or primary granules were not demonstrable as basophilic bodies.

Butterfish, *Poronotus triacanthus* (Peck). The primary granule is characteristically present in neutral red preparations (Fig. 8, *b*) and induction of new granules proceeds very slowly. The chondriosomes are usually granular. No long filamentous forms were observed. The reticular patterns and numbers of immature cells are about the same as in the scup (Fig. 8, *a*, *c*, and *d*).

Variegated minnow, *Cyprinodon variegatus* Lacépède. The primary neutral red bodies usually appear as single or double bodies and induction of secondary bodies occurs slowly. The mitochondria are predominantly of the granular type and are relatively numerous (Fig. 11, *a* and *b*). In the majority of the cells the reticular material is very scanty (Fig. 11, *c* and *d*), but in a few cells complete perinuclear "wreaths" of reticular material were seen. Occasional younger cells, possessing large nucleoli and dense reticulations, were also noted. The primary neutral red bodies of mature cells did not give a basophilic reaction with Wright's stain, but in many polychromatic cells they could be distinctly seen as basophilic granules.

Mummichogs, *Fundulus heteroclitus* (Linnæus) and *F. majalis* (Walbaum). The behavior of the erythrocytes is essentially alike in these two species. The primary neutral red bodies are characteristically present (Fig. 12, *b* and *c*). The reticular substance in general is scanty, appearing as radially arranged filaments (Fig. 12, *d*). A few

cells in the " wreath " stage and an occasional young cell with a large nucleolus were seen. The primary granules were not demonstrated by Wright's method.

Common eel, *Anguilla rostrata* (Le Sueur). The neutral red bodies are very minute, almost at the limit of visibility (Fig. 5, *a* and *d*). However, they gradually increase in size on exposure to the dye and secondary granules slowly appear (Fig. 5, *b*). The mitochondria are few in number and chiefly of the long, sinuous type. Brilliant cresyl blue reveals the presence of large numbers of immature erythrocytes, the reticular substance varying in distribution from complete " wreaths " to scattered isolated filaments and granules (Fig. 5, *c*). In stained smears many erythrocytes show varying degrees of cytoplasmic basophilia. The primary granules frequently appear as basophilic bodies in cells which stain orthochromatically in eosin or show a slight polychromasia. However, they were not distinguishable in more basophilic cells.

Sand dab, *Hippoglossoides platessoides* (Fabricius). The primary vital granules are readily demonstrated as single or double granules (Fig. 9, *a*). With longer exposure to the dye secondary granules, usually grouped in clusters, quickly appear (Fig. 9, *c* and *d*). Chondriosomes of both filamentous and granular types are present. Practically all the cells are mature, showing a very sparse reticulation (Fig. 9, *b*). An occasional cell with an incomplete open-meshed " wreath " was seen. A few basophilic erythrocytes were demonstrated with Wright's stain. The primary granules were frequently seen as basophilic bodies in erythrocytes which exhibit a slight polychromasia but could not be distinguished in mature cells.

Group II

In the erythrocytes of the fishes of this group the primary granules are not limited to a single or double unipolar body but are relatively numerous and have a perinuclear distribution.

Menhaden, *Brevoortia tyrannus* (Latrobe). In the menhaden the granules are very small, frequently barely visible at a magnification of nine hundred diameters. The granules are either arranged in a single definite line about the nucleus or scattered somewhat irregularly throughout the cytoplasm (Fig. 10, *a* and *b*). The irregular distribution of granules appears to be characteristic of the oldest cells. On longer staining with neutral red they increase in both size and number. Mitochondria of both the filamentous and granular types are present in restricted numbers.

All stages of maturating erythrocytes were encountered, but the majority of the cells were fully differentiated. The reticular patterns

vary all the way from a dense compact granular mass filling the entire cell to the mature condition in which only fine particles and scattered, short filaments are present (Fig. 10, *c* and *d*). In stained smears erythrocytes in the different stages of differentiation are clearly shown and the numerous perinuclear primary granules are seen distinctly as basophilic bodies in both polychromatic and mature cells.

Alewife, *Pomolobus pseudoharengus* (Wilson). The erythrocytes of the alewife are essentially like those of the menhaden in all their reactions to the dyes, but more immature cells are present (Fig. 14). Many of the younger cells contain large, clear, refractile globules which appeared as vacuoles in stained smears.

Mackerel, *Scomber scombrus* Linnæus. The erythrocytes of the mackerel differ only slightly from those of the menhaden and alewife (Fig. 13). The primary neutral red patterns are alike. Mitochondria of the filamentous type predominate. More immature cells are present than in the alewife and the reticulation patterns are accordingly very variable. The red cells react rapidly with neutral red and many secondary granules may develop. The primary neutral red granules are demonstrated as basophilic bodies by Wright's stain, but much more care must be taken in the differentiation of the stain.

Smooth dogfish, *Mustelus canis* (Mitchill) and spotted skate, *Raja diaphanes* Mitchill. The vital staining reactions of the erythrocytes of both these forms have been described by several investigators. The granules appearing after exposure to neutral red are very large and numerous (Figs. 15 and 17). The mitochondria are usually long and filamentous. Induction of new granules occurs rapidly. There is a relatively high proportion of immature red cells, and the reticulation patterns are very variable. In old cells the reticular substance is greatly reduced, appearing as scattered particles and short filaments.

With brilliant cresyl blue both vital granules and reticulation patterns are demonstrated as in the teleosts, but on long standing the dye in the granules disappears while the reticular substance remains brilliantly stained. The reduction of the dye in the granules appears to be characteristic only of the elasmobranchs and has been previously reported by Jokl (1925). Although the granules are large and readily seen in fresh unstained preparations, they cannot be demonstrated in smears stained by Wright's method and do not give a basophilic reaction.

The Relative Degree of Differentiation of the Mature Erythrocytes of Vertebrates

In the running description of the findings in the erythrocytes of the different species of fish, the staining reactions described related particu-

larly to the predominating cells, presumably the mature ones. During the course of the study it became increasingly obvious that no granules, either preëxistent or induced, were present in young cells exhibiting a complete, dense reticulation pattern. In somewhat older stages the primary granules were readily demonstrated, but induction of new granules either did not occur or proceeded very slowly. This secondary reaction varied greatly in different species, but the general trend was the same in all. In more mature stages induction usually occurred more readily, but in some old, perhaps senile, cells, which gave practically no reticulation reaction with brilliant cresyl blue, the secondary response to vital dyes again decreased.

The terms " primary " or " preëxistent," and " secondary " or " induced " are used here without reservation to designate the granules which are under discussion, since the cumulative evidence gathered from studies on fishes and amphibians indicates that such a distinction is valid. The granules which stain so readily with neutral red are frequently seen as refractile bodies in fresh preparations, and in dry-fixed smears are frequently demonstrated as basophilic inclusions. In most cases the characteristic form and location of these elements make their identification easy when they are rendered visible by other than supravital methods. Dornesco and Steopoe (1930*a*, 1930*b*) have also successfully blackened these primary bodies with silver nitrate methods (Da Fano and Cajal) in both teleosts and elasmobranchs. Their demonstration by a silver method in the elasmobranch erythrocyte is significant, since in these cells the granules fail to give a basophilic reaction in carefully stained smears.

The reality of the secondary granules cannot be disputed since they can be observed as they arise within the cells. Accordingly we have to deal with three distinct morphological entities, at least as they are demonstrated by supravital methods. The question of their chemical constitution and relationships is a baffling one and apparently little further progress can be made in this regard until better methods of study are devised.

Nevertheless, the characteristic reactions to vital dyes may be legitimately used as evidences of the progressive changes which occur within the differentiating erythrocyte, and such reactions therefore constitute useful criteria for determining the degree of differentiation reached by the mature erythrocytes of a given species. The criteria of maturity in one species are not necessarily valid in every detail for erythrocytes of another species, since the pictures obtained by vital dyes may vary in different groups of animals. Maturity of erythrocytes in general can best be defined as the stage at which the cell acquires its maximum

concentration of hemoglobin, and in normal animals cells of this type should predominate in the circulation. The degree of concentration of hemoglobin in mature cells in a given species is relatively constant and is closely correlated with the acquisition of characteristic staining reactions, but similarity of staining patterns in different species does not by any means indicate that the different erythrocytes have acquired the same concentration of hemoglobin. In other words, the erythrocytes of the different vertebrates are mature at varying levels of differentiation, the latter being measured by such staining reactions as are usually regarded as evidences of immaturity in the most highly differentiated red cells.

During the differentiation of the vertebrate erythroblast a striking series of changes occurs. Some are readily demonstrated in fixed preparations while others are adequately revealed only by supravital staining. Most of these changes are common to the erythrocytes of all vertebrates, but in the mammals an extreme degree of specialization is encountered. During differentiation the nuclear-cytoplasmic ratio changes greatly, the nucleoli undergo a gradual involution and may disappear, the distribution of chromatin in the nucleus is modified, and the basophilia of the cytoplasm is gradually lost and replaced by the eosinophilia of the hemoglobin. Also the volume of mitochondrial substance is progressively reduced, and frequently the form of the individual elements is changed.

The gradual reduction of the reticular substance, definitely correlated with a decreasing basophilia of the cytoplasm, is the most striking feature of the maturing erythrocytes when seen in supravital preparations. Less conspicuous changes involve the appearance and behavior of both types of granular inclusions, preëxistent and induced. Primary granules are usually absent from very young cells and disappear in a later but somewhat variable stage of differentiation. When they first appear they do not give a basophilic reaction in stained smears, but later in many instances they are characteristically basophilic. In mature cells of some animals the basophilic reaction is subsequently lost and the bodies are again demonstrated only by supravital methods. Moreover, in some vertebrates the primary granules may entirely disappear while in others they persist and assume characteristic patterns of distribution. Other granules of similar morphology and behavior may appear in cells that have stood in preparations for some time. Apparently induction of granules can occur only after some degree of differentiation of the erythrocyte has been attained, but the ability to respond in this manner may persist until full maturity is reached, and in many instances induced granules may appear even after the primary granules are no longer in evidence. In the mammals, however, the nucleus and all the cytoplasmic inclusions eventually disappear completely.

In the different vertebrates each of these changes in the maturing erythrocyte may take place at a different rate and to a varying degree. Accordingly, with the exception of the mammals, it is not always easy to decide which cells represent at maturity the more fully differentiated stage. In all vertebrates below the mammals the cells are characteristically nucleated with a few striking exceptions in the Amphibia (Emmel, 1924). In all of these animals the basophilia is eventually replaced by eosinophilia. The nucleoli may disappear; the nuclear-cytoplasmic ratio undergoes considerable changes, the nucleus becoming condensed and acquiring a characteristic chromatic pattern. That is, in ordinary stained smears the mature nucleated erythrocytes of vertebrates appear essentially alike except that they vary greatly in size. However, with the more delicate methods of supravital staining, quite striking differences are brought out. Accordingly, the patterns of granulation and reticulation afford the best criteria of the degree of differentiation of these cells, and of these two criteria the degree of persistent reticulation is probably the better since the reticular material is continuously present in the cell while the granules have a variable and much more complicated history. Still, the latter criterion cannot be entirely disregarded in comparing the nucleated red cells of the vertebrates.

Adequate data for the comparison of the erythrocytes of vertebrates are available for fishes and amphibians, but the blood of reptiles and birds has not been studied so extensively by means of vital dyes. In order to secure first-hand information concerning the conditions in the reptiles and birds, supravital preparations of blood from the painted turtle, horned toad, fence lizard (*Sceloporus undulatus*), and the common fowl were studied.

In the fishes (both elasmobranchs and teleosts) and birds little reticular substance is present in the mature cells (Fig. 18, *a*, *c*, and *d*), being represented mostly by scattered filaments and granular fragments. My observations on the fowl differ from those of Doan, Cunningham, and Sabin (1925), who report that the final stages of reticulation consist in a few discrete bodies stainable with vital dyes. These bodies are readily demonstrated by low concentrations of either neutral red or brilliant cresyl blue (Fig. 18, *b*), but when brilliant cresyl blue in sufficiently high concentration to stain the nuclear reticulum is used, scattered fragments and short filaments are also uniformly present in the cytoplasm. Brilliant cresyl blue is apparently reduced to some extent in the cell and the demonstration of persisting fragments of reticulum is possible only when the dye is present in considerable excess. In the amphibians and reptiles, on the other hand, a definite, more or less complete reticular pattern can be demonstrated in the mature erythrocytes, but in most

instances the amphibian erythrocytes contain the greater amount of reticular substance (Figs. 16, 19, 20, and 22). If the degree of persistence of reticulation is regarded as evidence of the degree of differentiation attained by the mature erythrocyte, it must be concluded that the red blood cells of fishes and birds are relatively more highly differentiated than those of the amphibians and reptiles.

The amount of material demonstrable as so-called primary vital granules in the mature erythrocytes of the several classes of vertebrates cannot be readily correlated with the degree of persistent reticular substance, but the history of the vital granules indicates that their presence in red blood cells is in some degree a measure of relative maturity. It has been already pointed out that the reticular substance is at a maximum in the young cells and gradually decreases as the erythrocytes mature, while in general the granular substance is not present at all until the cells are partially mature.

It is practically impossible to make any significant generalizations regarding the vital granules. The erythrocytes of each species must be considered separately if any accurate conclusions are to be drawn regarding relative maturity of the cells. It has been held by many that the granular material is but a phase of the filamentous reticulum. In very young cells the reticular substance is definitely granular before acquiring the filamentous form. The granular form is doubtless dependent on its concentration within the cell and is the result of the agglutinating or precipitating effect of the vital dye. The primary granules are, however, definite, discrete bodies, frequently distinguishable in fresh unstained cells, and may often be demonstrated in fixed material. They are apparently associated in some way with cell metabolism, and when the erythrocytes are exposed to a penetrating dye it accumulates first of all in these preformed structures. Whether the secondary or induced bodies are derived from reticular material is less easy to determine. But certain lines of evidence would appear to indicate that they are the result of a specific reaction of the cytoplasm to an excess of dye and do not represent local, sharply delimited accumulations of reticular material (Chlopin, 1927).

The history of the behavior of these three elements in the maturing erythrocytes further suggests that they are separate entities. In elasmobranchs the primary granules of mature cells are large and numerous when the reticular substance is reduced to the same degree as in the teleosts. In the amphibians the history is also variable, granules being either present or absent, depending on the species (Arrigoni, 1908; Beams, 1930; Dawson and Charipper, 1929; DeRoo and Ufford, 1930; Goda, 1929; Hibbard, 1928; Jordan, 1925; Stolz, 1928), while the

reticular material persists as a fairly complete, open network. In *Triturus viridescens* the erythrocytes rarely contain any granules either primary or secondary, but as Nigrelli (1929) has shown, the reticulation in mature cells is relatively abundant (Fig. 16). Other similar examples could be cited.

In the reptiles the reticulation is quite abundant in mature cells but the primary granules are single in the alligator, horned toad, and fence lizard, and clustered at one pole in the painted turtle. The granules in these several species can also be demonstrated as basophilic bodies in dried smears stained by Wright's method (author's observations), and the question of their being induced bodies cannot be raised. In the common fowl and pigeon, Doan, Cunningham, and Sabin (1925) regard the single vital granule as a vestige of the reticular substance. Forkner (1929) also states that in the domestic fowl the cytoplasm of the erythrocytes contains, after staining with neutral red, from none to several small, reddish brown bodies which are usually near the nucleus but often move about and may be far out near the cell border. These bodies are not demonstrated in smears stained by Wright's method but, as pointed out earlier, they have probably been demonstrated by other methods and mistaken for centrosomes. Their close resemblance to similar structures in teleosts and reptiles which can be shown to be primary bodies lends strength to the view that they are preëxistent in the fowl erythrocytes, and are not produced by a reaction of the dye with remnants of the reticular substance. In view of the evidence accumulated from a study of the nucleated erythrocytes of vertebrates, the author is inclined to accept the conclusion of Michels (1931) that the reticular substance in reticulocytes has no genetic relation to the vital granules of the mature red cells, but would disagree with his acceptance of the view that vital granules are surface structures, either precipitates of the stain or stained precipitates of the plasma. After a study of the irregular behavior of the vital granules in nucleated erythrocytes, it does not seem surprising that such granular cytoplasmic inclusions do persist even after the nucleus, chondriosomes, and reticular substance have disappeared from the mammalian cell. The appearance of primary granules in the cytoplasm is apparently a constant phenomenon in the maturation of the erythrocytes. It is only in the mammalian erythroplastid that they uniformly completely disappear and in this instance they mark the acme of erythrocyte differentiation. They, however, are not always the last vestige of immaturity to disappear, since in some higher urodeles and several anurans they disappear while the reticular substance is still present in relatively large amounts.

The appearance of secondary or induced granules in erythrocytes

following exposure to vital dyes apparently has only a very limited relation to the degree of differentiation attained by such cells. It is true that young cells with a high concentration of reticular substance react slowly and to a very limited degree to dyes, but the amount of reaction obtained in more mature cells is also very variable and seems not to be directly determined by the degree of differentiation attained. Rather the reaction appears to be species specific and to depend on the permeability of the cell and other factors inherent in the cytoplasm of the given species. Also the degree of the reaction with the cytoplasm cannot be correlated with the presence of a certain amount of reticular substance, since as much induction may occur in mature erythrocytes of fishes with a minimum of reticulation as in those of amphibians and reptiles where the reticular substance persists in greater amounts. The induction phenomenon in cells containing primary granules is usually confined at first to the areas of the cell containing such preëxistent bodies. In the early stages of induction the primary granules themselves become enlarged and new granules then appear in their immediate vicinity. Later new bodies may form irregularly throughout the cytoplasm.

The phenomenon of induction in erythrocytes appears to be closely related to the "krinome" reaction to vital dyes described by Chlopin (1927) for many other cells of the animal body. Freely penetrating stains such as the basic dyes appear to accumulate within the cytoplasm of the red blood cells and appear first in the preformed bodies when they are present. Later, as more dye is accumulated, it is segregated by some reaction of the cytoplasm into newly formed structures. In some erythrocytes this accumulation and subsequent segregation of dye within the cytoplasm appears to continue progressively and to surprising limits, while in other erythrocytes the reaction proceeds only to a minimum extent. As has been already noted, many of the external factors influencing this reaction are known, but the factors within the cytoplasm, which are apparently of utmost importance, are unknown. The reaction, as in the anurans (Beams, 1930), proceeds as well in mature cells without preformed bodies as in cells in which preformed bodies are numerous and conspicuous. The final loss of the ability to react, as in the most highly differentiated mammalian erythrocytes, would seem to indicate that the degree of differentiation attained by the cell in some way determined or limited the cytoplasmic reaction to the dye. Such a conclusion, however, is rendered more or less untenable by the irregular behavior of the nucleated erythrocytes of other vertebrates, whose degree of differentiation at maturity can be estimated by the degree of persistence of reticulation. In such cells, except in very young stages,

there is no significant correlation between the relative degree of differentiation attained and the degree of neo-formation of vitally stained bodies.

Before concluding this discussion of the relative degree of differentiation of the mature vertebrate erythrocytes, one other morphological feature of maturing erythrocytes should be mentioned, although the observations on it are not all comprehensive. It has already been noted that with relatively high concentrations of brilliant cresyl blue the nucleoli in immature cells of the fishes appeared as deep blue bodies in a light blue, apparently homogeneous nucleus, the nuclear reticulum not being shown with such concentrations of the dye. The nucleoli (plasmosomes) are relatively large in young cells and vary in number, but there are seldom more than three in any cell. They grow smaller as the erythroblasts differentiate and usually appear as single, small, spherical bodies. After the stage at which the reticular substance appears as an open-meshed, almost complete network the nucleoli are more rarely seen, and in the mature cells containing scattered fragments of reticulum they are usually absent. In the catfish, however, nucleoli are uniformly present in the mature erythrocytes.

Nucleoli are not readily demonstrated in the mature cells of the Amphibia by brilliant cresyl blue, but they may be stained if concentrations of dye sufficiently high to bring out the chromatin reticulum of the nuclei are used. The dye, however, must not be intense enough to stain the chromatin a dark blue or the nucleoli are obscured (Fig. 16). In such preparations of the erythrocytes of *Necturus* and *Triturus* the nucleoli, numbering from one to four, appear as dark blue bodies lying between the coarse bars of light blue chromatin. They are somewhat irregular in form and are not so sharply outlined as in the fishes. At first it seemed doubtful if these bodies were nucleoli, but a comparison of the mature cells with younger stages in the circulation appears to establish their plasmosomal nature.

In the reptiles studied (horned toad, fence lizard, and painted turtle) for this feature of the mature erythrocyte, the nucleolus is uniformly found as a single spherical body in all mature erythrocytes, and is a striking feature of all properly stained preparations (Figs. 19, 20, and 22). Immature cells were rarely encountered in the blood of these animals and no comparisons with the nucleoli of younger erythrocytes were made. In the blood of the fowl no nucleoli could be distinguished in mature cells; but in an occasional immature cell, still in the stage with a more or less complete reticular net, small single spherical nucleoli were observed.

This method of demonstrating nucleoli, supravitally with brilliant

cresyl blue, in red blood cells does not appear to have been previously employed. It seems to be a delicate method and to give clear pictures of nucleoli even when they cannot be easily demonstrated in fixed and stained preparations. In many amphibian erythrocytes brilliant cresyl blue also stains the achromatic contents of the nucleus a reddish violet, while the chromatin is a light blue and the nucleoli deep blue. In such cells the nucleoli appeared to be imbedded in the achromatic substance.

These observations on the persistence of the nucleoli are correlated in a very satisfactory manner with the findings on the degree of persistence of reticular material and afford additional evidence that the mature erythrocytes of fishes and birds are relatively more highly differentiated than those of amphibians and reptiles.

The degree of differentiation of erythrocytes of the several classes of vertebrates as determined by the criteria of persistent reticulation and presence of nucleoli also seems to correlate fairly well with the size of these cells. The Amphibia as a class have the largest erythrocytes (Fig. 21). Reptiles have blood cells next in size and fishes come next, then birds and mammals. It is difficult, however, to conceive that cell size could directly influence the degree of persistent reticulation or the persistence of nucleoli. The presence of primary granules might possibly be dependent to some extent on this factor, since the products of metabolism might be less readily eliminated from larger cells and temporary accumulations be segregated in the cytoplasm in the form of granules.

Summary

The reactions of the mature erythrocytes of seventeen species of fishes to the vital dyes neutral red, Janus green B, and brilliant cresyl blue, have been studied. In most teleosts the primary vital granules are readily demonstrated by neutral red and consist of one or two small granules eccentrically placed near one pole of the nucleus, but in the menhaden, alewife, and mackerel the primary granules are most numerous and are either arranged in a single definite line about the nucleus or scattered irregularly throughout the cytoplasm. In the elasmobranchs the granules are large, numerous, and scattered. In a majority of the teleosts the primary granules may be demonstrated as basophilic bodies in dry films stained by Wright's method and are also frequently seen in fresh unstained preparations.

Secondary or induced granules may also appear in the cytoplasm of cells exposed for long periods to the dye. The degree of induction of new bodies does not appear to depend entirely on external factors but is determined to a large extent by factors inherent in the cytoplasm of the

given species. In general the mitochondria are filamentous, reduced in number, and lie in close contact with the surface of the nucleus.

The reticular substance in all mature erythrocytes of the fishes is greatly reduced and appears either as short irregular filaments or as minute granular remnants. It is best demonstrated with brilliant cresyl blue.

An attempt is made to compare the relative degree of differentiation attained by the mature erythrocytes of the several classes of vertebrates. The following criteria have been considered: changes in nuclear-cytoplasmic ratio; chromatin distribution in the nucleus; involution of the nucleoli; loss of basophilia; changes in the form, distribution, and volume of mitochondrial substance; reduction of reticular substance; amount of primary granules; and degree of induction of secondary granules. Of these criteria the degree of persistence of reticulation has been found to be the most consistent, and on this basis the several classes of vertebrates are arranged in the following ascending order of relative differentiation attained by their erythrocytes at maturity: amphibians, reptiles, fishes, birds, and mammals. This arrangement is also supported by the behavior of the nucleoli, which persist in the erythrocytes of amphibians and reptiles but are not usually demonstrated in the mature cells of fishes and birds.

The history of the primary and secondary granules is less regular and consequently less useful for measuring the relative differentiation attained by the cells of different classes of vertebrates. However, within a given class of vertebrates it is concluded that the presence of a large number of primary granules or the rapid induction of new granules in mature cells may be regarded as supplementary evidence of a lesser degree of differentiation. The presence of primary granules or the degree of induction of new granules, however, cannot always be correlated with the degree of persistence of reticulation.

It is also concluded on the basis of this survey of the vertebrate erythrocyte that primary granules, secondary granules, and patterns of reticulation as revealed by vital dyes, must be regarded as three separate entities which are not genetically related.

LITERATURE CITED

ARRIGONI, C., 1908. Ueber die Metamorphose des Kernes der menschlichen Erythroblasten und über die Natur der chromatophilen Substanz der Erythrozyten. *Folia Haem.*, **6**: 444.

BEAMS, H. W., 1930. The So-called 'Segregation Apparatus' of the Erythrocyte of Frog and Necturus Blood. *Anat. Rec.*, **47**: 341 (abstract).

BHATTACHARYA, D. R., AND F. W. R. BRAMBELL, 1925. The Golgi Body in the Erythrocytes of the Sauropsida. *Quart. Jour. Micros. Sci.*, **69**: 357.

BREMER, L., 1895. Ueber das Paranuclearkörperchen der gekernten Erythrocyten, nebst Bemerkungen über den Bau der Erythrocyten in Allgemeinen. *Arch. f. mikr. Anat.*, **45**: 433.

BREMER, L., 1895. Die Indentität des Paranuclearkörperchens der gekernten Erythrocyten mit dem Centrosom. *Arch. f. mikr. Anat.*, **46**: 618.

CHLOPIN, N. G., 1927. Experimentelle Untersuchungen über die sekretorischen Prozesse im Zytoplasma. I. Über die Reaktion der Gewebselemente auf intravitale Neutralrotfärbung. *Arch. f. exper. Zellforsch.*, **4**: 462.

DAWSON, A. B., 1928. The 'Segregation Apparatus' of the Amphibian Erythrocyte and Its Possible Relation to the Golgi Apparatus. *Anat. Rec.*, **39**: 137.

DAWSON, A. B., 1929. A Further Study of the Reaction of the Amphibian Erythrocyte to Vital Dyes, Osmic Acid, and Silver Salts, with Special Reference to Basophilia and Reticulation. *Anat. Rec.*, **42**: 281.

DAWSON, A. B., 1930. Chondriome and Vacuome of the Differentiating Erythrocyte of Necturus and Their Relation to the So-called Golgi Substance of Erythrocytes. *Anat. Rec.*, **46**: 281.

DAWSON, A. B., 1931. Supravital Studies on the Erythrocyte of the Catfish (Ameiurus nebulosus, Lesueur), with Special Reference to the Nittis stigma. *Anat. Rec.*, **49**: 121.

DAWSON, A. B., AND H. A. CHARIPPER, 1929. A Comparative Study of the Amount and Distribution of the Neutral-Red Bodies in the Erythrocytes of Urodeles. *Anat. Rec.*, **43**: 299.

DEHLER, A., 1895. Beitrag zur Kenntnis des feineren Baues der roten Blutkörperchen beim Hühnerembryo. *Arch. f. mikr. Anat.*, **46**: 414.

DEROO, G. I., AND E. H. UFFORD, 1930. An Investigation of the Staining Reactions of Erythrocytes of the Leopard Frog to Nile-blue Sulphate, with Special Reference to the Nature of the Segregation Apparatus and Golgi Substance. *Anat. Rec.*, **46**: 297.

DOAN, C. A., R. S. CUNNINGHAM, AND F. R. SABIN, 1925. Experimental Studies on the Origin and Maturation of Avian and Mammalian Red Blood-Cells. Contributions to Embryology, Vol. 16, *Pub. Carnegie Inst.*, No. 361, pp. 163–227.

DORNESCO, G. TH., ET J. STEOPOE, 1930*a*. L'appareil de Golgi des globules rouges des Téléostéens marins. *Compt. rend. Soc. Biol.*, **105**: 288.

DORNESCO, G. TH., ET J. STEOPOE, 1930*b*. L'appareil de Golgi des hématies des Sélaciens. *Compt. rend. Soc. Biol.*, **105**: 446.

EISEN, G., 1897–1899. Plasmocytes; the Survival of the Centrosomes and Archoplasm of the Nucleated Erythrocytes, as Free and Independent Elements in the Blood of Batrachoseps attenuatus Esch. *Proc. Calif. Acad. Sci.*, Ser. 3, Zool., Vol. 1.

EISEN, G., 1899. On the Blood Plates of the Human Blood with Notes on the Erythrocytes of Amphiuma and Necturus. *Jour. Morph.*, **15**: 635.

EMMEL, V. E., 1924. Studies on the Non-nucleated Elements of the Blood. II. The occurrence and genesis of non-nucleated erythrocytes or erythroplastids in vertebrates other than mammals. *Am. Jour. Anat.*, **33**: 347.

FERRARI, R., 1930. Sur la formation de l'hémoglobine dans le noyau des érythroblastes de grenouille. *Arch. Ital. Biol.*, **84**: 44.

FORKNER, C. E., 1929. Blood and Bone Marrow Cells of the Domestic Fowl. *Jour. Exper. Med.*, **50**: 121.

GIGLIO-TOS, E., 1896. Sur les cellules du sang de la Lamproie. *Arch. Ital. Biol.*, **26**: 93.

GODA, T., 1929. Cytoplasmic Inclusions of Amphibian Cells with Special Reference to Melanin. *Jour. Fac. Sci., Imp. Univ., Tokyo*, Sec. IV, Zool., Vol. 2, pp. 51–122.

GOLGI, C., 1920. Il centrosoma dei globuli rossi del sangue circolante dell'uomo e di altri animali. *Haem. Arch. Ital.*, Anno 1, pp. 333–359.

HIBBARD, H., 1928. Contribution à l'étude de l'ovogenèse, de la fécondation, et de l'histogenèse chez Discoglossus pictus Otth. *Arch. de Biol.*, **38**: 251.

JOKL, A., 1925. Über vitalfärbbare Erythrozytengranulationen („ Substantia metachromatico-granularis ") beim Rochen, nebst weiteren Bemerkungen über das Blut dieser Tiere. *Zeitschr. f. mikr.-anat. Forsch.*, **2**: 461.

JOLLY, J., 1903. Origine nucleaire des paranuclei des globules sanguins du Triton. *Compt. rend. l'Assoc. d. Anat.*, **5**: 115.

JORDAN, H. E., 1925. A Study of the Blood of the Leopard Frog, by the Method of Supravital Staining Combined with the Injection of India Ink into the Dorsal Lymph Sac, with Special Reference to the Genetic Relationships among Leucocytes. *Am. Jour. Anat.*, **35**: 105.

KNOLL, W., 1931. Weitere Beiträge über die Entstehung des Hämoglobins im Erythroblastenkern. *Folia Haem.*, **44**: 310.

LEWIS, M. R., AND W. H. LEWIS, 1926. Transformation of Mononuclear Blood-cells into Macrophages, Epithelioid Cells and Giant Cells in Hanging-Drop Blood Cultures from Lower Vertebrates. Contribution to Embryology, Vol. 18, *Pub. Carnegie Inst.*, No. 363, pp. 95–120.

MICHELS, N. A., 1931. The Erythrocyte. A critical review of its normal and pathological morphology and physiology with data on the normal red cell count and technic. *Haematologica*, Vol. 2.

NIGRELLI, R. F., 1929. Atypical Erythrocytes and Erythroplastids in the Blood of Triturus viridescens. *Anat. Rec.*, **43**: 257.

NITTIS, S., 1930. A Surface Structure (?) in Normal Nucleated Erythrocytes. *Anat. Rec.*, **46**: 365.

SABRAZÈS, J., ET L. MURATET, 1900. Granulations mobiles des globules rouges de l'hippocampe. *Act. Soc. Linn. Bordeaux*, **55**: lxv.

SABRAZÈS, J., ET L. MURATET, 1900. Corpuscles mobiles endoglobulaires de l'hippocampe. *Compt. rend. Soc. Biol.*, **52**: 365.

SABRAZÈS, J., ET L. MURATET, 1900. Granulations mobiles dans les globules rouges de certains poissons. *Compt. rend. Soc. Biol.*, **52**: 415.

SABRAZÈS, J., ET L. MURATET, 1902. Granulations endoglobulaires des globules rouges des anguilles jeunes. *Act. Soc. Linn. Bordeaux*, **57**: cix.

SABRAZÈS, J., ET L. MURATET, 1908. Observations sur le sang de la torpille (Torpedo marmorata Risso). *Act. Soc. Linn. Bordeaux*, **62**: cxiii.

SABRAZÈS, J., ET L. MURATET, 1908. Le sang de l'axolotl. Granulations du cytoplasme: origine nucléolaire. *Folia Haem.*, **6**: 171.

SCOTT, G. H., 1928. A Method for Making Permanent Preparations of Supravitally Stained Blood Cells. *Anat. Rec.*, **38**: 233.

STOLZ, R., 1928. Le granulazioni basofile degli eritrociti nei vertebrati inferiori. *Atti Soc. Ital. Sci. Nat. Mus. Civ. (Milano)*, **67**: 93.

STOLZ, R., 1928. Ematopoiesi normale e sperimentale nei pesci teleostei. *Haematologica*, **9**: 419.

URTUBEY, L., 1927. Sobre la fijación del azul de metileno por el vacuoma en los eritrocitos de " Pleurodeles waltlii." *Arch. de Card. y Hemat.*, **8**: 390.

VILLA, L., 1930. Sull' origine dell' emoglobina. *Arch. d. Fisiol.*, **28**: 233.

VON APATHY, ST., 1897. Protokollauszug der am 2. April 1897 abgehaltenen naturwissenschaftlichen Fachsitzung der medizinisch naturwissenschaftlichen Sektion. *Sitzgsber. d. med.-naturw.* Sektion des Siebenburg. Museumsvereins. Jahrg. 22 II, naturw. Abt.

WEIDENREICH, F., 1903. Die roten Blutkörperchen. I. *Erg. der Anat. Entwickl.*, **13**: 1.

YOFFEY, J. M., 1929. A Contribution to the Study of the Comparative Histology and Physiology of the Spleen, with Reference Chiefly to its Cellular Constituents. I. In fishes. *Jour. Anat.*, **63**: 314.

EXPLANATION OF PLATES

All drawings are from dry preparations and were outlined at the same magnification by means of a camera lucida. The details were filled in free-hand from sketches of the fresh cells. Erythrocytes stained with brilliant cresyl blue to demonstrate patterns of reticulation are shown with solid nuclei. All others, with the exception of Fig. 21, were stained either with neutral red and Janus green B or neutral red alone. The mitochondria are usually filamentous but some granular forms are present. Ordinarily they may be distinguished by their juxta-nuclear position.

PLATE I

Explanation of Figures

1. Erythrocytes of the toadfish showing, (*a*) induction of granules and reticulation; (*b* and *c*) primary granules and mitochondria; (*d*) primary granules and reticulation.

2. Erythrocytes of the sea robin showing, (*a* and *b*) the primary granules and mitochondria; (*c*) induction of granules after twenty minutes; (*d*) reticulation.

3. Erythrocytes of the sea bass showing, (*a*) primary granules and mitochondria; (*b*) induction of granules after twenty minutes; (*c*) reticulation in a mature cell; (*d*) reticulation in an immature cell which possesses a nucleolus.

4. Erythrocytes of the tautog showing, (*a* and *b*) primary granules and mitochondria; (*c*) reticulation in an immature cell; (*d*) reticulation in a mature cell.

5. Erythrocytes of the eel showing, (*a* and *d*) primary granules and mitochondria; (*b*) induction of granules after twenty minutes; (*c*) reticulation in a mature cell.

6. Erythrocytes of the cunner showing, (*a* and *b*) primary granules and mitochondria; (*c* and *d*) reticulation in mature cells.

7. Erythrocytes of the scup showing, (*a* and *c*) primary granules and mitochondria; (*b*) reticulation in a mature cell; (*d*) reticulation in an immature cell.

8. Erythrocytes of the butterfish showing, (*a*) reticulation in a mature cell; (*b*) primary granules and mitochondria; (*c* and *d*) reticulation in immature cells.

9. Erythrocytes of the sand dab showing, (*a*) primary granules and mitochondria; (*b*) granules and reticulation; (*c* and *d*) induction of granules after twenty minutes.

10. Erythrocytes of the menhaden showing, (*a*) perinuclear primary granules and mitochondria; (*b*) granules after twenty minutes; (*c*) reticulation in a mature cell; (*d*) reticulation in an immature cell.

11. Erythrocytes of *Cyprinodon* showing, (*a* and *b*) primary granules and granular mitochondria; (*c* and *d*) reticulation in mature cells.

12. Erythrocytes of *Fundulus majalis* showing, (*a*) induction of granules after twenty minutes; (*b* and *c*) primary granules and mitochondria; (*d*) reticulation in a mature cell.

13. Erythrocytes of the mackerel showing, (*a*) reticulation in an immature cell; (*b*) perinuclear primary granules and mitochondria; (*c*) induction of granules after twenty minutes; (*d*) reticulation in a mature cell.

14. Erythrocytes of the alewife showing, (*a*) induction of granules after twenty minutes; (*b*) reticulation in a mature cell; (*c*) primary granules and mitochondria; (*d*) reticulation in an immature cell.

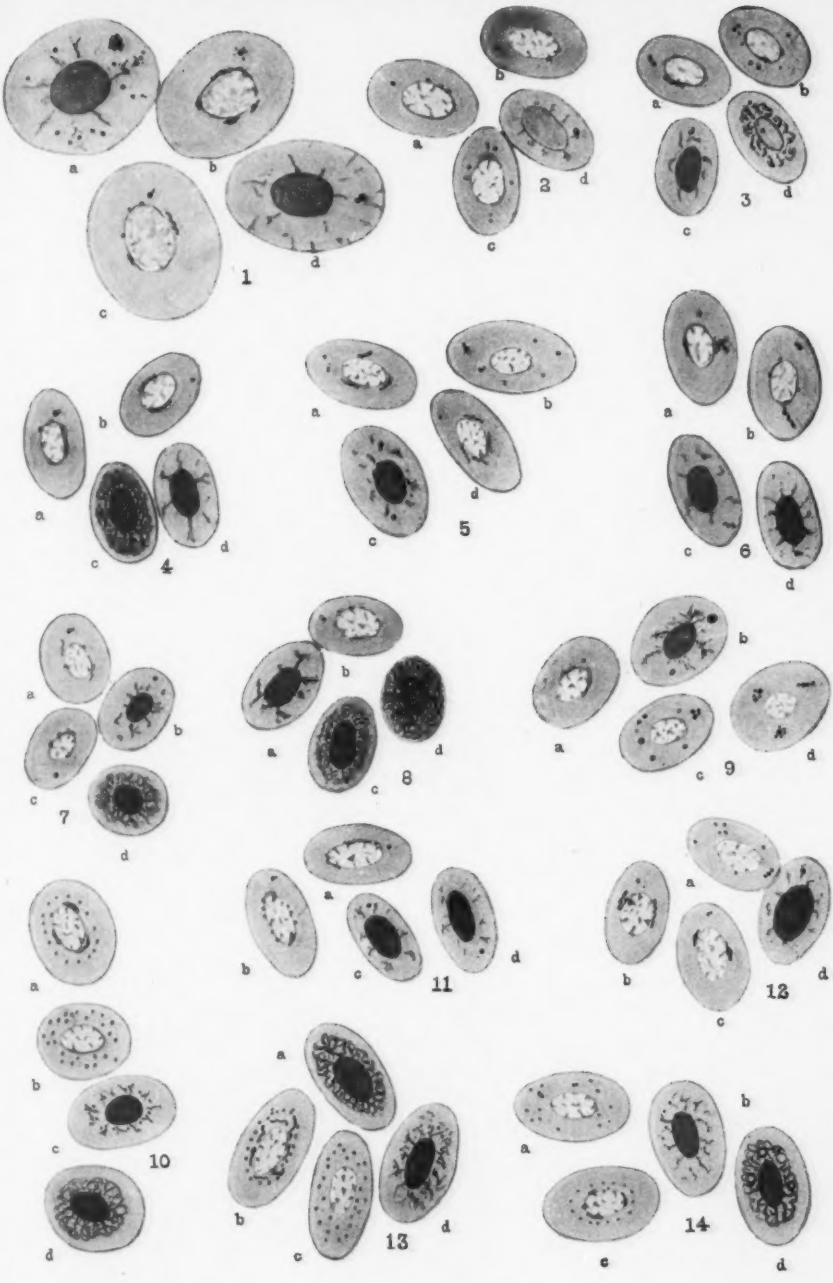
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PLATE II

Explanation of Figures

15. Erythrocytes of the smooth dogfish showing, (*a*) the primary granules and mitochondria; (*b*) reticulation in a mature cell; (*c*) reticulation in an immature cell.

16. An erythrocyte of *Triturus viridescens* showing reticulation of a mature cell. Note the complete absence of granules.

17. Erythrocytes of the spotted skate showing, (*a, b,* and *c*) primary granules and mitochondria; (*d*) reticulation in a mature cell.

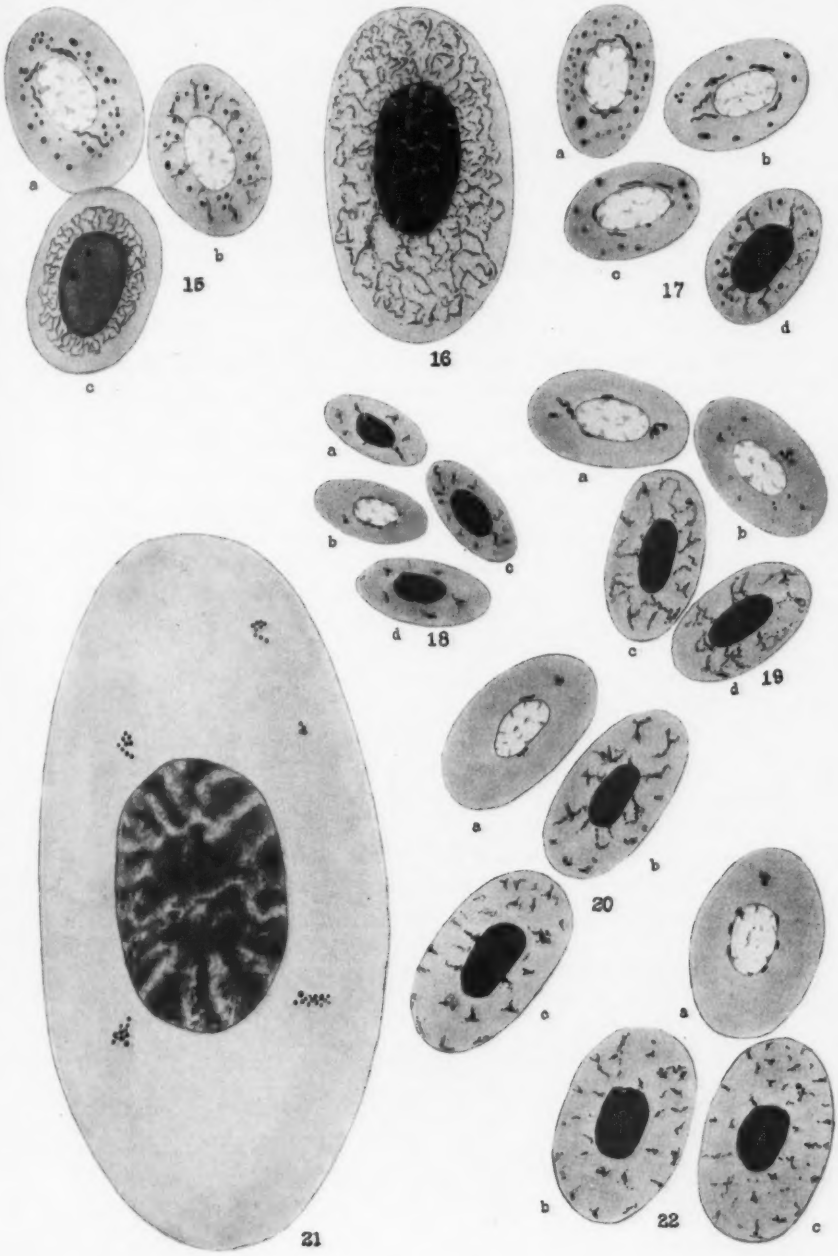
18. Erythrocytes of the domestic fowl showing, (*a, c,* and *d*) reticulation of mature cells; (*b*) primary granules and mitochondria.

19. Erythrocytes of the fence lizard showing, (*a*) primary granules and mitochondria; (*b*) induction of granules after twenty minutes; (*c* and *d*) reticulation and nucleoli in mature cells.

20. Erythrocytes of the horned toad showing, (*a*) primary granule and mitochondria; (*b* and *c*) reticulation and nucleoli in mature cells.

21. An erythrocyte of *Amphiuma means* from a smear stained by Wright's method, showing the primary granules as clusters of basophilic bodies.

22. Erythrocytes of the painted turtle showing, (*a*) the primary granules and mitochondria; (*b* and *c*) reticulation and nucleoli in mature cells.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CYCLOTRICHIUM MEUNIERI SP. NOV. (PROTOZOA, CILIATA); CAUSE OF RED WATER IN THE GULF OF MAINE

PHILIP B. A. POWERS

ZOÖLOGICAL LABORATORY, UNIVERSITY OF PENNSYLVANIA

I. INTRODUCTION

During the warmest days of August, 1931, there were noticed in Frenchman Bay [1] on several occasions, great patches and lanes of red water. This has been known to occur in previous years but rarely so early as the fifth of August. These patches of red water were caused by swarms of small red animals, which appeared in layers from one to three meters beneath the water level. In spots these animals would be more crowded, thus varying the density of the patch as a whole and making the color vary from a brick to a blood red. Between the action of the wind and tide these patches would be streaked out in great lanes and irregular areas. On some days these patches would be more numerous than on others. As to the origin and ultimate fate of these areas of red water, the writer has no information.

A microscopic examination of a drop of sea water containing these organisms revealed hundreds of small red animals which moved rapidly in a zig-zag fashion reminding one of the characteristic movements of *Halteria*. On preliminary examination their appearance suggested that of trochophore larvæ; however, their incredible numbers and small size suggested that they were protozoa. Since the living animals died and disintegrated within one or two minutes upon exposure in a drop of water on a glass slip, no definite conclusion could be reached as to their morphology from a study of the living material.

Further study indicated that these animals were swarms of a ciliate belonging to the genus *Cyclotrichium* Meunier. I have named it *Cyclotrichium meunieri* sp. nov.

The writer wishes to express his appreciation to Mr. William Procter of Bar Harbor, Me., in whose laboratory this study was initiated; and to Dr. D. H. Wenrich for his many helpful suggestions and criticism of the manuscript.

[1] Frenchman Bay is that part of the Gulf of Maine which separates the northwest side of Mt. Desert Island from the mainland.

II. Technique

Quart jars of this red water were collected and allowed to stand in a cold place for about 20 minutes, in which time the organisms settled to the bottom. With a long pipette this sediment was collected and spurted into flasks half filled with warm (40° C.) fixative. Both Schaudinn's and Bouin's fixatives were used. After 30 minutes' fixation the animals were removed from the fixative with the aid of a centrifuge, washed and stored in 70 per cent alcohol. At the close of the summer, vials containing this fixed material were brought back to the University, where the slides were made and the study completed.

By mixing, on a cover-glass, a drop of this alcoholic sediment of fixed organisms with an equal amount of Mayer's egg albumen, spreading it carefully so that it would dry slightly, and finally flooding the cover-glass with absolute alcohol, these minute organisms were fastened to the cover-glass to facilitate their easy manipulation during staining. After 15 minutes in absolute alcohol the cover-slips were gradually transferred to water. Part of the material was stained with iron hæmatoxylin, Mayer's hæmalum, or Delafield's hæmatoxylin; and part by Feulgen's nucleal reaction.

It was found necessary to section some of this material, and in order to handle these small animals in paraffin a mixture of the alcoholic sediment and Mayer's egg albumen was placed in a concavity cut in a small block of preserved (70 per cent alcohol) liver, coagulated with absolute alcohol; and this block, with embedded protozoa, was then handled as a piece of tissue. By infiltrating in 67° paraffin and treating the block with ice water before cutting, sections of 4 μ could be cut with ease. The same stains were used for the sectioned material as for the whole mounts, with the exception that counterstains of orange G or eosine were used to demonstrate the cilia.

III. Observations

Cyclotrichium meunieri (Fig. 1), is almost oval, with the anterior end blunted and the posterior region slightly conoid.

Size.—This ciliate is relatively small. Twenty-five specimens selected at random from fixed material averaged 33 μ (25–42 μ) in length and 22 μ (18–34 μ) in width through the anterior region, whose diameter is somewhat greater than that of the posterior portion.

Cytostome.—The cytostome could not be definitely located; however, most of the specimens show, at the larger end, a depression (Figs. 1 and 2) slightly funnel-shaped and leading into the interior without any well-marked structures. This location of the cytostome agrees in general with that described by Meunier (1910) for *C. cyclokaryon;* and

with descriptions given of the cytostomal regions of a number of species of *Cyclotrichium* described by Fauré-Fremiet (1924).

Ciliary Band.—A broad band of cilia is found about the middle. The fine, closely-set cilia are from 5 to 6 μ long and are found only in the depressed mid-region or ciliary band. Besides being depressed and ciliated, this band is further indicated by 52–60 striations which run parallel to the longitudinal axis. Each striation is made up of 5 to 9 granules; anteriorly the granules form a definite ring (Figs. 1 and 3, *A. G. R.*), while posteriorly they become slightly larger (Fig. 1). With iron hæmatoxylin these granules stain black, as do the metaplasmic granules of the endoplasm. With Delafield's hæmatoxylin or Mayer's hæmalum the metaplasmic granules fail to stain while the granules of the *ciliary band* are definitely outlined, thus indicating that the two types of granules are not the same. Because of the close association

EXPLANATION OF PLATE

Cyclotrichium meunieri sp. nov. All drawings have been made at a magnification of 2900 diameters and reduced about two-fifths in printing. The animals have been fixed with Bouin's fluid, stained in Heidenhain's hæmatoxylin, with the exception of those shown in Figs. 7 and 8 which were treated for the Feulgen reaction.

Abbreviations

A.G.R., anterior granular ring
B.G., basal granules
C., cilia
C.B., ciliary band
C.Bu., ciliary bundles
C.C., compound cilia
Ch., chromatin
C.P., chromatophore platelets
Cy., cytostome
E., endoplasm with inclusions
E.V., extra-nuclear vesicle
L.S., longitudinal striations
M., macronucleus
m., micronucleus
Mc., metaplastids
P., pellicle
P.B., pyrenoid body
Pl., plastin
S.S., shrinkage space or artifact

Explanation of Figures

1. Side view of *Cyclotrichium meunieri*. Chromatophore platelets and pyrenoid bodies do not stain in the whole mounts.

2. Sagittal section showing arrangement of chromatophore platelets, pyrenoid bodies, etc.

3. Sagittal section through region of ciliary band showing the arrangement of the basal granules.

4. A diagram of the possible arrangement of cilia in compound units to explain structures as shown in Fig. 6.

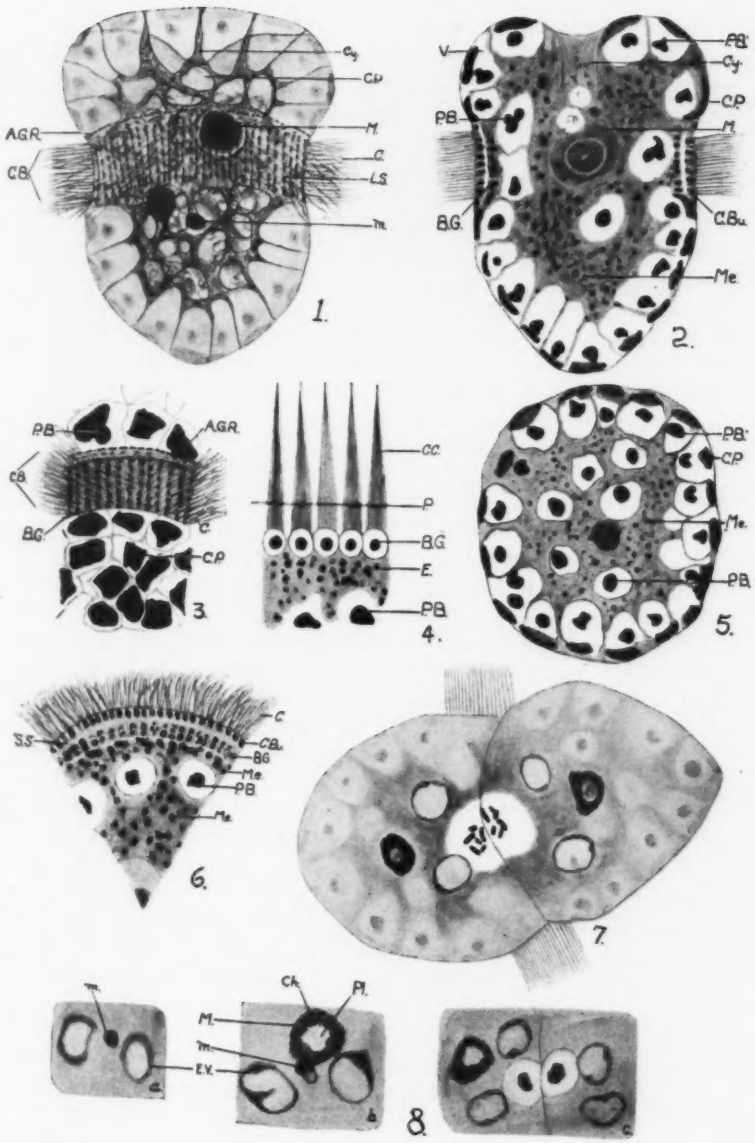
5. Cross section through anterior region.

6. Cross section through region of ciliary band. Cilia are finer and more numerous than could be shown in drawing.

7. Early telophase in dividing individual; six chromosomes are seen in each daughter.

8. Nuclear complexes showing variations. (*a*) micronucleus and two extra-nuclear vesicles; (*b*) micro- and macronucleus and two extra-nuclear vesicles; (*c*) a stage somewhat later than that shown in Fig. 7. Macronucleus is seen in only one of the daughter cells.

PLATE I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of these granules of the striations with the cilia, I have designated them as basal granules.

The uneven movement of the living animal suggests the presence of cirri or membranelles; however, since the sectioned material showed only these exceedingly fine and numerous cilia and their large associated (compound?) basal granules (Fig. 3, *B. G.*), it would seem that we have in the living condition a system of compound cilia which at death separate into their component units (Fig. 6, *C.*). A diagrammatic interpretation of these compound cilia is shown in Fig. 4. Further evidence for this arrangement is presented by the staining reaction. With iron hæmatoxylin the points of insertion of the cilia stain after the manner of a clump of fibrils (Fig. 6, *C., Bu.*), suggesting definite bunches or bundles of cilia.

Ectoplasm.—Beneath a thin, smooth pellicle, completely shielding the endoplasm, is a series of irregularly concave chromatophore platelets (Fig. 2, *C. P.*), each with an associated pyrenoid body (Fig. 2, *P. B.*); the whole being inclosed by a large vacuole. The red color of *C. meunieri* is doubtless due to the presence of a hæmatochromatous substance localized in the chromatophore platelets, which are possibly of an amylaceous nature. These platelets stain deeply with iron hæmatoxylin but not at all with Mayer's hæmalum. That these platelets have a definite body is demonstrated by the fact that they are often torn from their place due to the impact of the knife during sectioning. In the region of the ciliary band the platelets are missing while the pyrenoid bodies with their vacuoles remain (Fig. 2).

Endoplasm.—After staining sections with iron hæmatoxylin the endoplasm is crowded with many darkly staining bodies. These are designated metaplasmic granules, for they are doubtless associated with the metabolic processes. Because of the abundance of these metaplasmic granules, the nuclear apparatus could be demonstrated successfully only by the aid of the Feulgen technique.

A typical ciliate nuclear complex is present. The macronucleus is slightly irregular and demonstrates a definite core of plastin (Fig. 8*b*, *M.*) surrounded by a layer of granular chromatin. The micronucleus is small, sometimes vesiculated (Fig. 8*b*, *m*). Besides the macro- and micronucleus there are found one or more bodies, irregular in shape and staining but slightly with Feulgen's reagent, which are designated as extra-nuclear vesicles (Fig. 8, *E. V.*). These extra-nuclear vesicles are always present and seem to be either the formative or degenerative stage in the development of the macronucleus.

A number of dividing individuals were studied, and in one (Fig. 7) showing an early telophase stage in the division of the micronucleus six

chromosomes could be counted in each daughter nucleus. A somewhat later stage than this is shown in Fig. 8*c*; in this case only one daughter individual received a definitive macronucleus, the other had but the extranuclear vesicles.

Occurrence.—Since single individuals of *C. meunieri* have been found in sea water taken either from the storage tanks of the laboratory, or among the material from plankton hauls; it seems reasonable to look upon this species as a member of the protozoan fauna of this region. Its sudden appearance in swarms among the surface plankton of the bay must be correlated with the periodic enrichment of the water by the nitrogen-bearing algæ whose numbers increase during periods of warmth and excessive sunshine; all of which factors tend to make areas of the bay excellent culture media for these red water ciliates. In the present instance the summer had been warmer than usual, particularly during the last of July and the first week of August.

IV. Discussion

Many organisms are known, under favorable conditions, to multiply in such numbers as to discolor great bodies of water. Martin and Nelson (1929) review this subject and give instances of red water occurring in Delaware Bay due to the swarming of *Amphidinium fusiforme*. Kofoid and Swezy (1921) record the occurrence of swarms of *Gonyaulax polyhedra* as being the most frequent cause of red water along the Pacific coast.

I have placed the ciliate causing red water in the Gulf of Maine in the genus *Cyclotrichium* Meunier because it seems to resemble very closely a ciliate of rare occurrence in the plankton hauls from Barents Sea. Meunier (1910) established this genus for *C. cyclokaryon* but figured a number of ciliates as *Cyclotrichium* Sp? because their poor preservation would not permit further classification. Those ciliates were included in the genus *Cyclotrichium* which had a ciliary band or belt in an equatorial depression which divided the body into two subspherical halves. Making allowance for the poor fixation of his material, the organisms which he described under the name of *Cyclotrichium* Sp? may be likened to those found off the coast of Maine which I have named *C. meunieri.*

The Maine fishermen recognize this red water as a source of food for the herring sardine and it is said that when the red water is present in the herring's intestines they become unfit for sale. Mackerel are also reported by the fishermen as sometimes containing "red feed" in their digestive tracts. It would seem that "red water" and "red feed" are due to two different organisms. Inquiring into this condition fur-

ther, the writer corresponded with Dr. A. G. Huntsman, Director of the Atlantic Biological Station at St. Andrews, mentioning these occurrences. Dr. Huntsman replied as follows:

"In the St. Andrews region we have never seen the water coloured crimson by the form you mention. In warm summers the warmest strip near the center of the upper end of Passamaquoddy Bay sometimes becomes decidedly reddish and this has been found due to large numbers of different kinds of Tintinnoids which have been studied by Professor J. N. Gowanlock, although his report has not yet been published. In the St. Andrews region the term 'red feed' is given to copepods as they occur in the stomach of herring, particularly the young herring or sardine. The most abundant form is *Calanus* and at times the swarms of this species give a reddish cast to small areas of the water."

To my knowledge *Cyclotrichium meunieri* is the first holotrichous ciliate to be associated with the appearance of red water in the ocean.

V. Summary

1. Red water in Frenchman Bay is caused by the swarming of a small red ciliate, *Cyclotrichium meunieri* sp. nov.

2. This organism is ovoid, about 33 μ long, and has in a wide depression about its middle a band of fine cilia. It has been suggested that compound cilia may be present in the living animals. The endoplasm is well shielded by a peripheral series of chromatophore platelets, each with an associated pyrenoid body.

3. Six chromosomes were observed in each of the daughter micronuclei in an early telophase of division.

Literature Cited

Fauré-Fremiet, E., 1924. Contribution a la Connaissance des Infusoires Planktoniques. Paris.

Kofoid, C. A., and Olive Swezy, 1921. The Free-Living Unarmored Dinoflagellata. Memoirs of the University of California, Volume 5, p. 44.

Kudo, R. R., 1931. Handbook of Protozoology. Chas. C. Thomas, Pub.

Martin, G. W., and Thurlow C. Nelson, 1929. Swarming of Dinoflagellates in Delaware Bay, New Jersey. *Bot. Gaz.*, **88** (*2*) : 218–224.

Meunier, A., 1910. Microplankton des mers de Barents et de Kara. Duc D'Orléans, Campagne Arctique de 1907. Vol. I, p. 168. Vol. II, Pl. xvii, Fig. 7; Pl. xx, Fig. 15, 16, 17; Pl. xxiii, Fig. 9.

THE NUMBER OF PRE-ADULT INSTARS, GROWTH, RELATIVE GROWTH, AND VARIATION IN DAPHNIA MAGNA

BERTIL GOTTFRID ANDERSON [1]

BIOLOGICAL LABORATORY, WESTERN RESERVE UNIVERSITY

Until recently growth and variation in Crustacea have been studied by means of preserved materials. These materials may consist of a single collection taken in a certain locality at a specific time or several collections made in different localities at different times. Often conclusions are drawn from an isolated collection. Such studies necessarily neglect the past history of the individual. Any conclusions reached by such methods regarding growth and variation can only be regarded as tentative and subject to verification by experiments with individually reared animals.

As a result of such studies on *Coronis,* Brooks (1886) was led to the statement: " . . . the length of the larva increases uniformly at each moult by one fourth of its length before the moult." Later Fowler (1909) designated this numerical relation " Brooks' Law." He also called the fixed fractional increase the " growth factor."

That " Brooks' Law " does not hold for Cladocera has been shown by Rammner (1930) after a series of studies on individually reared animals from several genera. Gurney (1929) doubts that the above relations exist for copepods. Other arthropods as well do not follow " Brooks' Law." Calvert (1929) found that larval Odonata grow quite irregularly.

Such studies with Cladocera have perhaps led to the conclusion that individuals of a species pass through a definite number of pre-adult instars. When a graph of a population is made wherein the number of individuals of a size class is plotted against size, *i.e.,* total length, a series of size modes is secured (Fig. 1). These size modes are taken as representative of the growth stages of the organism. Usually females of a given species which are of a specific size or larger bear eggs in their brood chambers. The number of size modes between the embryonic stages and the mode of the smallest egg-bearing females is taken as the number of pre-adult instars for the species.

[1] A part of the experimental work related in this paper was done at the Zoölogical Laboratory of the State University of Iowa.

Inspection of graphs of size distributions such as in Fig. 1 shows that the size groups represented by the modes are not entirely distinct. The size groups in the lower size ranges are usually quite distinct but not decidedly disjoined from each other. Those in the higher size ranges

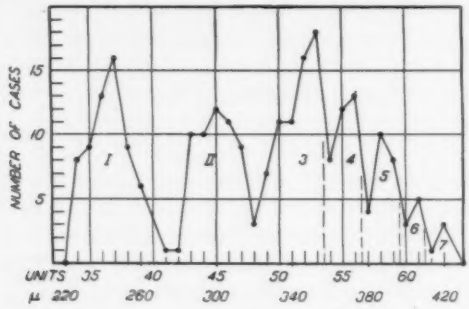


FIG. 1. Distribution of a population of *Chydorus sphæricus* according to length (after Werner).

tend to run together and are not so easily identified. Since the size groups are not entirely distinct over the pre-adult range and the past history of each individual is not known, the conclusion that all indi-

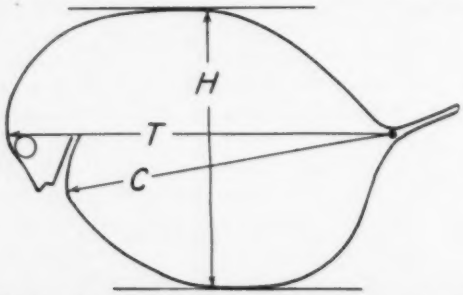


FIG. 2. Diagram showing methods of making measurements. *T*—total length, longest dimension of animal exclusive of spine. *C*—carapace length, longest dimension of carapace exclusive of spine. *H*—height, the shortest distance between two lines tangent to the carapace, as illustrated, and parallel to the line of *T*. This measure of height is affected but little by the number of young in the brood chamber.

viduals of a species have a definite number of pre-adult instars is not necessarily valid.

The writer finds that the number of pre-adult instars for *Daphnia magna* is rather variable. Calvert (1929) reported that the number of larval instars for certain species of Odonata is not constant. Dover (1931) reported that the number of moults in *Orgyia turbata* decreases

when fed on *Crotolaria*. Singh-Pruthi (1925, etc.) found that the number of moults for mealworms can be varied. Schubert (1929) examined *Ceriodaphnia reticulata* from two ponds. The material from the one pond was collected early in July, 1926. He believed that all *C. reticulata* in this collection were primiparous in the fourth instar. The material from the second pond was taken in mid-September of the same year. His findings regarding the latter were that 60 per cent of the individuals were primiparous in the third instar and that 16 per cent were still immature in the fourth. He concluded that at least two varieties of *C. reticulata* exist in these ponds. Apparently the number of pre-adult instars is variable in this species. Agar (1930) reports that *Simocephalus gibbosus* and *Daphnia carinata* have three and four pre-adult instars respectively but adds that a small percentage of the individuals become mature in one less than the average.

The aim of this paper is to present data on growth of individually reared *Daphnia magna* and further to consider the application of the equation

$$y = bx^k$$

to relative growth and variation in Cladocera.

Materials and Methods

Female *Daphnia magna* Straus. of several clones were employed. One clone was used for the major portion of the work. To determine whether or not the observed results were characteristic only of the one clone, some six others were tested. One of the latter was secured from Dr. A. M. Banta. All of the other clones, including the one first mentioned, were derived from ephippial eggs which may be traced to Banta's stocks.

Individual females were isolated within six hours of their release from the brood chambers of the mothers. These were measured and placed in separate vials containing thirty to thirty-five cubic centimeters of a culture medium. Banta's manure-soil medium (Banta, 1921) and oatmeal and wheat modifications were employed. More uniform results were obtained from the regular manure-soil medium than from modifications, as the results of one series of experiments indicate.

Where a modified culture medium was used, the animals were placed in old manure-soil medium and a drop of an oatmeal mixture or wheat infusion was added daily. Water was added to replace the fluid lost by evaporation. The oatmeal mixture was prepared by cooking rolled oats for a half hour in about twice the amount of water ordinarily used in making a porridge. The mixture was then strained through gauze and kept in a refrigerator until used. The wheat infusion was prepared by

TABLE I

Mean total lengths of female *Daphnia magna* of one clone during each of the pre-adult instars and the first adult for different classes of individuals. The classes are based on the number of pre-adult instars and the nature of the culture medium.

MEDIUM	MANURE-SOIL	OATMEAL MODIFICATION				
Number of Pre-adult Instars	5	5	6	7	8	
Number of Cases	19	8	29	39	5	
First Instar	0.85 ± 0.01	0.80 ± 0.01	0.84 ± 0.01	0.83 ± 0.01	0.77 ± 0.02	
Second Instar	1.09 ± 0.02	1.02 ± 0.01	1.00 ± 0.01	0.94 ± 0.01	0.86 ± 0.02	
Third Instar	1.39 ± 0.02	1.33 ± 0.03	1.17 ± 0.01	1.05 ± 0.01	0.99 ± 0.03	MEAN TOTAL LENGTH IN MM.
Fourth Instar	1.77 ± 0.02	1.70 ± 0.03	1.36 ± 0.02	1.19 ± 0.01	1.12 ± 0.04	
Fifth Instar	2.13 ± 0.02	2.06 ± 0.04	1.64 ± 0.02	1.38 ± 0.01	1.22 ± 0.05	
Sixth Instar	Adult 2.60 ± 0.02	Adult 2.56 ± 0.03	1.99 ± 0.02	1.69 ± 0.01	1.55 ± 0.10	
Seventh Instar			Adult 2.49 ± 0.02	2.08 ± 0.03	1.87 ± 0.09	
Eighth Instar				Adult 2.56 ± 0.03	2.20 ± 0.04	
Ninth Instar					Adult 2.52 ± 0.03	
Number of Young in First Brood	6.4 ± 0.3	6.4 ± 0.6	7.3 ± 0.2	8.6 ± 0.3	5.8 ± 0.8	
Number of Young in Second Brood	9.5 ± 0.5	9.4 ± 0.6	7.4 ± 0.3	7.1 ± 0.2	5.2 ± 1.0	

boiling wheat in water, decanting the fluid, and allowing this to stand open for several days before using. When the manure-soil medium was used exclusively, one-third of the fluid was removed semiweekly from each vial and replaced by fresh medium.

At the time of isolation of the individuals and after every moult each individual was placed in a watch glass together with a few drops of the culture medium. Just enough of a saturated solution of chloretone was added to bring about cessation of movement. The chloretone did not appear to have any detrimental effects. By means of an ocular micrometer the measurements as illustrated in Figure 2 were made. The three measurements consisted of total length exclusive of the spine, carapace length, and height. Camera lucida outline drawings were made during each instar for each of three animals. In addition to these measurements, note was taken as to the presence of eggs in the brood chamber. Within twenty-four hours after their release the young were removed from the vials and counted.

Size and shape change only at the time of moulting in this species. Repeated measurements of any dimension during a single instar always gave the same results. Agar (1930) pointed out this fact for *Daphnia carinata* and *Simocephalus gibbosus*. Such being the case, a single set of measurements suffice for each animal during any one instar.

All the experiments were carried out at room temperature (18°–23° C.).

Number of Pre-Adult Instars and Growth

The results of observations on the number of pre-adult instars, *i.e.*, the number of instars elapsing between the time of release of the individual female from the brood chamber of her mother and the appearance of eggs in its own brood chamber, and the mean total length of the animals during each are summarized in Table I. The data were segregated on the basis of the number of pre-adult instars and the culture medium used. The number of pre-adult instars varied for the animals reared in the oatmeal modification. For those reared on the standard manure-soil medium the number was constant. Twenty individuals only were used in the latter series, one of which died before reaching maturity.

The number of pre-adult instars above five may perhaps be explained on the basis of food deficiency. Great numbers of large ciliates were found in the modified medium. These probably reduced the number of smaller microörganisms that ordinarily would have been available as food to the *Daphnia*. The ciliates themselves were too large to be consumed. Conditions similar to this arose at a time when great numbers of the annelid *Aeolosoma* were found in the culture medium. At times

a culture medium seems to stimulate the adult females to produce great numbers of young, but this same lot of medium does not seem sufficient for the development of the young.

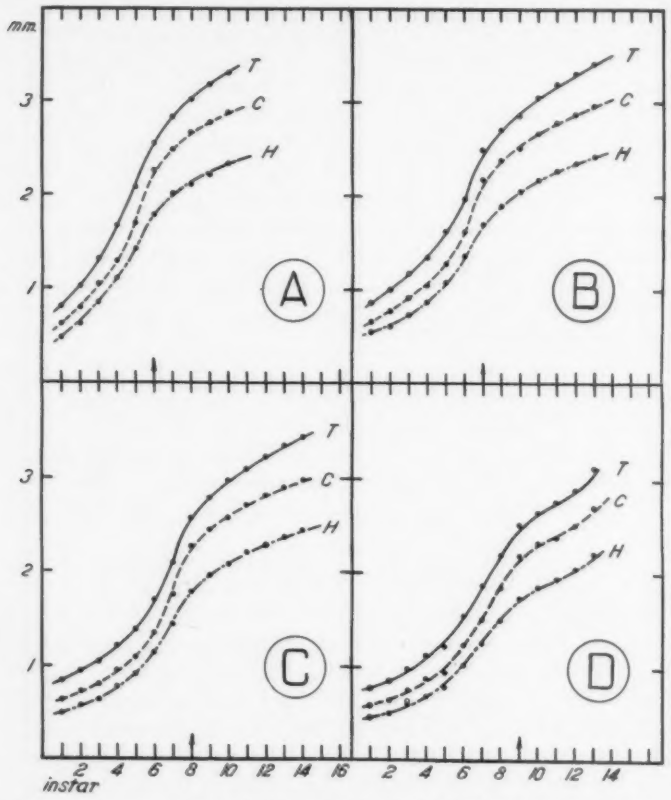


FIG. 3. Growth curves of different classes of individuals reared on the oatmeal modification; *A*—those animals with five pre-adult instars (7 individuals), *B*—six (18 individuals), *C*—seven (30 individuals), *D*—eight (5 individuals). *T*—total length; *C*—carapace length; *H*—height. The arrow indicates the first adult instar.

The animals used in the experiment with the modified medium were all released on the same day. The time taken to reach sexual maturity varied from six to ten days. The number of days corresponded approximately to the number of pre-adult instars in each case. The animals were all kept under identical conditions and the culture medium was from the same lot. The experiment using the standard manure-soil medium was begun several weeks later.

Table I brings out an interesting relation. The mean size for each group during the instar when eggs first appear in the brood chamber varies from 2.49 mm. to 2.60 millimeters. Apparently *Daphnia magna* must attain a certain size before becoming sexually mature. Singh-

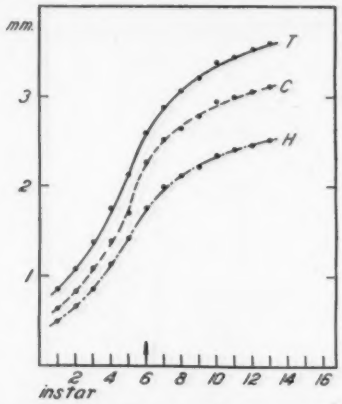


FIG. 4. Growth curves of a group of animals reared on unmodified manure-soil medium. These had five pre-adult instars (7 individuals). *T*—total length; *C*—carapace length; *H*—height. The arrow indicates the first adult instar.

Pruthi (1925, etc.) found that metamorphosis of mealworms took place only after the larvæ were full-grown.

Rammner (1930*b*) has found that eggs were produced after the fourth instar in *Daphnia magna*. After observation on well over a thousand individually reared females, the writer has never observed less than five. L. A. Brown has observed five pre-adult instars for this

TABLE II

Number of Individuals Primiparous During the Sixth to Ninth Instar

Clone	Sixth Instar	Seventh Instar	Eighth Instar	Ninth Instar	∞
B	4	10	4	2	0
C	8	3	1	0	1
D	7	4	0	0	0
E	9	1	1	0	0
F	6	11	2	0	0
G*	10	4	4	0	1

∞ Those living beyond the 10th instar without bearing eggs.
* Clone secured from Dr. A. M. Banta.

species (unpublished data). Rammner's smaller number may be due to some factor in the culture medium or perhaps to a genetic difference in his animals.

Figures 3 and 4 are growth curves constructed from data secured from animals included in Table I. The data used were taken only

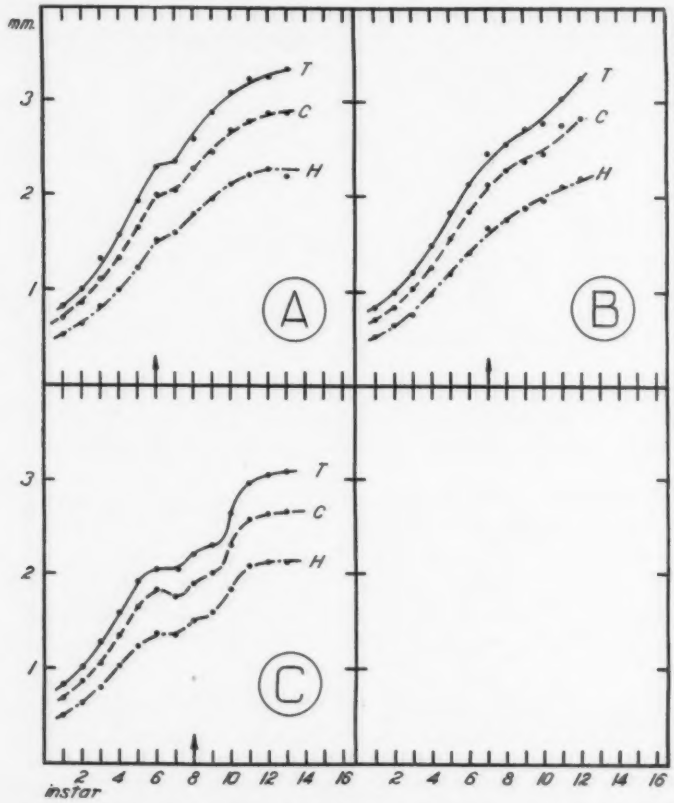


FIG. 5. Growth curves of three broodmates of clone *B*. Animal *A* had five pre-adult instars; *B*, six; and *C*, seven. *T*—total length; *C*—carapace length; *H*—height. Arrow indicates first adult instar.

from those animals which were still living during the last instar recorded on the graphs. All such are included. The inflection in the curves always occurs at a point which corresponds to the time of sexual maturity.

All the individuals used in the above experiments were of the same

clone. To determine whether or not the variation in the number of pre-adult instars was characteristic only of this clone, another series of experiments was performed using six other clones. Five of these were raised from ephippial eggs. A sixth clone was secured from Dr. A. M. Banta. Manure-soil medium and the wheat modification were employed in these tests. Table II gives the results. Examination of this

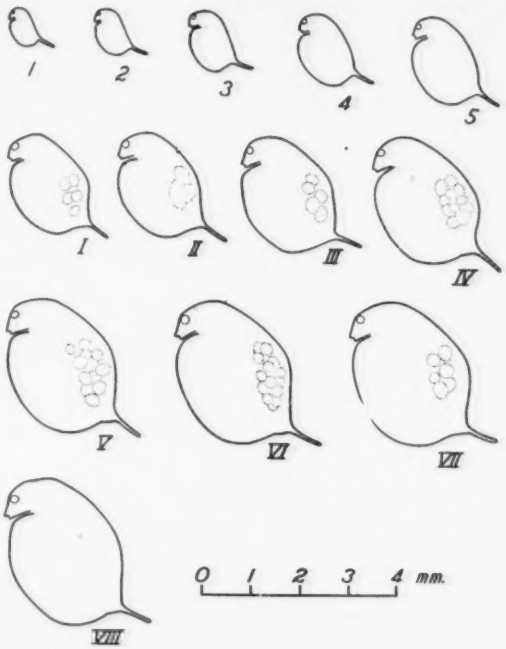


FIG. 6. Outline drawings of a single animal during each of the first thirteen instars. The animal is the one for which growth curves are shown in Figure 5*A*. The arabic numerals designate pre-adult instars; the Roman numerals—adult instars.

table shows that the variability in the number of pre-adult instars is characteristic of all clones used. Variation occurred to about the same extent in both media.

Two of the animals included in the above experiments lived for at least thirteen instars without bearing young. Ordinarily the ovary in the adult assumes a green color, especially toward the end of the instar. Microscopic examination did at no time reveal the development of the green color in the ovaries of either animal. In one of them growth did not seem retarded. Her total length during the thirteenth instar equaled

the average total length, during the same instar, of those primiparous in the sixth.

Individual growth curves for three brood mates of clone *B* are shown in Figure 5. Each of these as designated *A*, *B*, and *C*, bore its first clutch of eggs in the sixth, seventh, and eighth instars respectively. These were raised under the same conditions and at the same time, using the same lot of unmodified manure-soil medium. The measure-

TABLE III

Growth ratios for various classes of animals according to the nature of the culture medium, number of pre-adult instars, and clone

Instars	CLASS *							
	M6	M7	M8	M9	R6	B6	B7	B8
1– 2	1.27	1.16	1.13	1.11	1.26	1.24	1.19	1.25
2– 3	1.29	1.17	1.10	1.15	1.29	1.28	1.21	1.23
3– 4	1.27	1.14	1.17	1.14	1.27	1.19	1.23	1.24
4– 5	1.25	1.21	1.14	1.09	1.22	1.23	1.23	1.21
5– 6	1.24†	1.21	1.22	1.27	1.22†	1.19†	1.18	1.07
6– 7	1.10	1.26†	1.24	1.21	1.11	1.02	1.15†	1.00
7– 8	1.07	1.09	1.23†	1.18	1.07	1.10	1.04	1.08†
8– 9	1.05	1.06	1.08	1.15†	1.05	1.11	1.07	1.05
9–10	1.04	1.07	1.06	1.05	1.05	1.07	1.02	1.15
10–11		1.05	1.04	1.05	1.02	1.05	1.09	1.12
11–12		1.03	1.04	1.05	1.02	1.01	1.07	1.03
12–13		1.03	1.05	1.08	1.02	1.02		1.01
13–14			1.03					
Number of Animals	7	18	30	5	7	1	1	1

* Classes—M6, M7, M8, and M9 from clone *A* reared on modified medium and primiparous in the sixth, seventh, eighth, and ninth instars respectively. R6 from clone *A* and reared on the regular manure-soil medium, primiparous in the sixth instar. B6, B7, and B8 from clone *B*, primiparous in the sixth, seventh, and eighth instars respectively. This table is based on the same data as are Figs. 3, 4, and 5. The ratios are based on total length.

† Denotes instar during which animals were primiparous.

ments for these were made by means of camera-lucida drawings. Figure 6 is a reproduction of the series of camera-lucida drawings for animal *A*.

The irregularities in the number of pre-adult instars and in the growth of *Daphnia magna* as brought out above indicate that no law of growth such as "Brooks' Law" is valid under all circumstances. Table III brings the case out more clearly. This table gives the values of the ratio of the total length during each instar for each group to that

of the previous instar. These values correspond to the " Wachstums-quotienten " of Rammner (1930) and others. For those cases in which the individuals were primiparous in the sixth instar the value is fairly

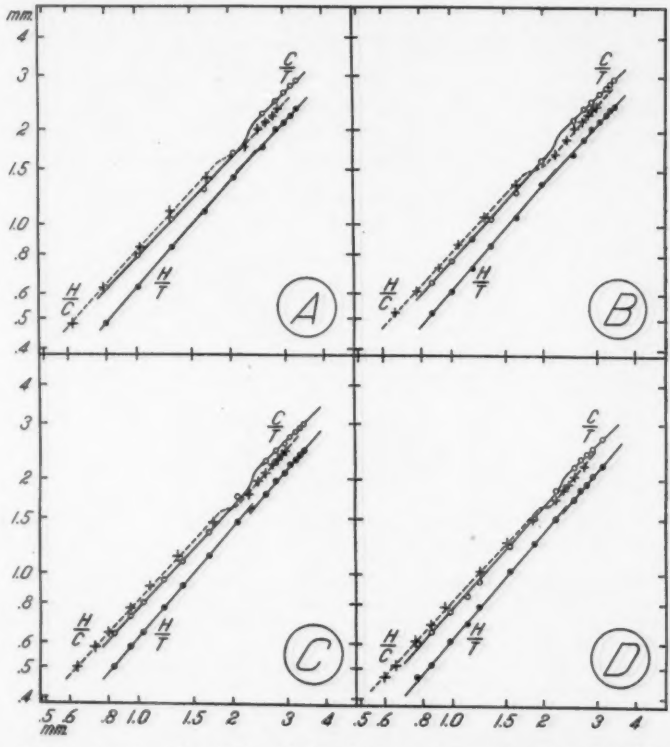


FIG. 7. *Log log* plots of the relations between carapace length and total length C/T, height and total length H/T, and height and carapace length H/C during each instar for different classes of animals reared on the oatmeal modification—the same for which growth curves are shown in Figure 3. The lines were drawn according to the calculated values of the constants given in Table IV. The breaks in the relations are coincident with sexual maturity. *A*—animals with five pre-adult instars (7 individuals), *B*—six (18 individuals), *C*—seven (30 individuals), and *D*—eight (5 individuals).

constant during the pre-adult stages. In all other instances the value is quite variable. After sexual maturity the value decreases considerably and approaches unity in old age. The validity of " Brooks' Law " with regard to *Daphnia magna* may therefore be considered to depend on the conditions of the individual and of the environment.

Relative Growth and Variation

The foregoing portion of this paper has brought out the wide irregularities in the general growth rate of individual *Daphnia magna*. In spite of these irregularities the relative growth of parts is quite constant. If the logarithm of the carapace length be plotted against the logarithm of the total length for each, two straight lines may be drawn—one through the points for the pre-adult instars and the other for the adult instars. The lines so drawn differ slightly in slope and in position (Figs. 7, 8, and 9). Such *log log* plots of height against total length

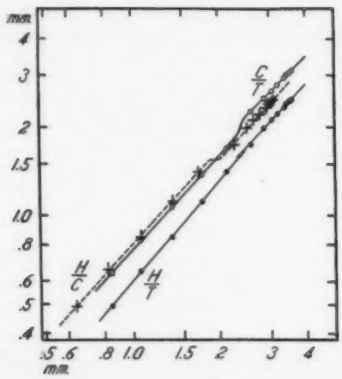


Fig. 8. *Log log* plots of the relations between carapace length and total length C/T, height and total length H/T, and height and carapace length H/C during each instar for a group of animals reared on unmodified manure-soil medium—the same for which growth curves are shown in Figure 4. The lines were drawn according to the calculated values of the constants given in Table IV. The breaks in the relations are coincident with sexual maturity.

and against carapace length show similar characteristics. These linear relations can be expressed as

$$y = bx^k,$$

where b is a constant and k is the " differential growth ratio " of y compared with x. Huxley (1924, etc.) [2] has found that a large number of organs obey this law. Hersh (1928, 1931) has shown that the facet numbers in the dorsal and ventral lobes of bar-eyed *Drosophila* conform to this equation. Robb (1929) has applied this relation to relative growth of organs in mammals and found it satisfactory.

[2] For an elucidating treatise on relative growth see Huxley " Problems of Relative Growth " (Methuen Co., London, 1932). This work became available to the author after having submitted the present paper to the editors.

TABLE IV

Values of the constants *b* and *k* during pre-adult and adult instars for various classes of *Daphnia magna* based on the nature of the culture medium, number of pre-adult instars, and clone. These relations are shown graphically in Figs. 7, 8, and 9.

	TOTAL LENGTH (x) CARAPACE LENGTH (y)				TOTAL LENGTH (x) HEIGHT (y)				CARAPACE LENGTH (x) HEIGHT (y)				NUMBER OF ANIMALS
	Pre-adult		Adult		Pre-adult		Adult		Pre-adult		Adult		
Class*	k	b	k	b	k	b	k	b	k	b	k	b	
M6	1.03	0.78	0.99	0.88	1.12	0.62	1.03	0.67	1.09	0.81	1.04	0.77	7
M7	1.07	0.77	0.96	0.91	1.15	0.62	1.07	0.65	1.08	0.82	1.12	0.72	18
M8	1.08	0.77	1.02	0.86	1.16	0.62	1.15	0.60	1.08	0.82	1.13	0.71	30
M9	1.08	0.77	0.95	0.92	1.14	0.62	1.14	0.61	1.06	0.81	1.20	0.68	5
R6	1.06	0.76	0.97	0.90	1.13	0.60	1.05	0.65	1.07	0.80	1.08	0.73	7
B6	1.01	0.84	1.02	0.85	1.01	0.63	1.06	0.64	1.00	0.75	1.04	0.76	1
B7	1.02	0.85	1.07	0.82	1.05	0.64	1.10	0.62	1.03	0.76	1.03	0.76	1
B8	1.07	0.83	1.01	0.86	1.10	0.61	1.05	0.66	1.03	0.75	1.05	0.77	1

* Classes—M6, animals of clone *A* raised on modified medium and primiparous during the sixth instar; M7, M8, M9, as M6 but primiparous during the seventh, eighth, and ninth instars respectively. R6, animals reared on the regular manure-soil medium and primiparous during the sixth instar. B6, B7, B8, individuals of clone *B* primiparous during the sixth, seventh, and eighth instars respectively. The classes are the same as those in Table III and in Figs. 3, 4, 5, 7, 8, and 9 and the values of the constants are based on the same data.

An interesting feature coming out of this study is that relative growth changes at sexual maturity. This may be seen on examination of any one of the *log log* graphs. The change is much more distinct in clone *A* (Figs. 7 and 8). The change in clone *B* is less discernible

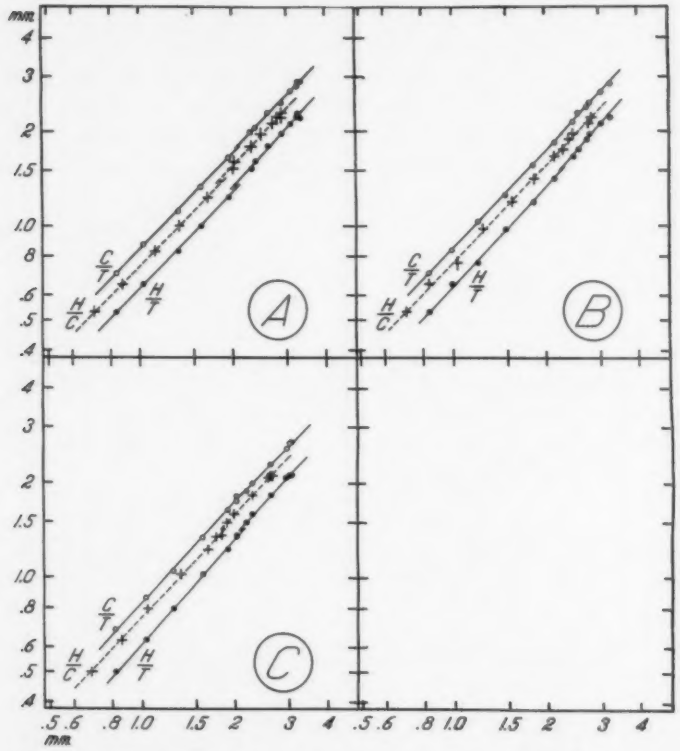


FIG. 9. *Log log* plots of the relations between carapace length and total length C/T, height and total length H/T, and height and carapace length H/C during each instar for three individual broodmates of clone *B*—the same for which growth curves are shown in Figure 5. The lines were drawn according to the calculated values of the constants given in Table IV. The breaks in the relations are coincident with sexual maturity. Animal *A* had five pre-adult instars, *B* six, and *C* seven.

(Fig. 9). Table IV gives the values of the constants b and k for both clones. These were computed by the method of averages. Since all points on any one graph represent the same number of cases, the cal-

culated values of the constants are practically as accurate as those secured by a more elaborate method.

Huxley (1927) has suggested that heterogony in *Maia* begins at sexual maturity. He also noted that this is probably true for male *Gammarus*. Examination of Figure 1 in the same report shows a change in relative growth of the large chela as against the rest of the body in *Uca pugnax*. Huxley makes no mention as to its significance. Robb (1929) has pointed out that a change takes place in the relative growth of various organs and suggests that another factor becomes involved. These changes are apparently coincident with sexual maturity.

Since the value of k approximates unity for all above relations, both for pre-adult and adult instars of *Daphnia magna*, and the value of b for each relation varies only over a small range, the animal does not change in proportions, to any great extent, during growth. Wesenberg-Lund (1926) writes that variations in *D. magna* of Denmark are insignificant. This condition is therefore as expected.

In a great many Cladocera pronounced variations occur. Rammner (1927) has discussed several methods for their study. As far as the writer is aware, no one has considered applying the equation

$$y = bx^k$$

in such studies.

Masses of data on variation have been collected by various workers on several species (Werner, 1924; Rammner, 1926; Heberer, 1928). The data as presented by these workers do not allow of the proper manipulation for testing the validity of the equation for the purpose. Were the original data available, a satisfactory test might be made. Rammner's (1928) data on individually reared *Chydorus sphæricus* and *Pleuroxus trigonellus* seem quite irregular. Woltereck's (1925) data on individually reared *Daphnia cucullata* seem satisfactory for the pre-adult instars. In *log log* plots of the head length against the body length a break in the relations appears in about the middle of the pre-adult instar range and another at sexual maturity. The first change seems likely to affect only the value of the constant b in the equation while k remains approximately the same. The second change would probably affect the values of both constants. The values of the constants for the adult instars cannot be determined, since the data includes measurements for only one and sometimes two adult instars. Different varieties give different values for the constants. The general trend of the relations is the same.

The advantage of applying the equation

$$y = bx^k$$

to data on variation in Cladocera is that the direction of variation may be expressed numerically in the constants b and k. The data employed could be taken from preserved material such as that used by Werner and others. Huxley and his coworkers have used this method in the study of heterogeny in Crustacea with apparent success. Collections from several localities, as well as those made in the same locality at different seasons, might thus be readily analysed and compared.

The author wishes to express his appreciation to Drs. J. H. Bodine of the State University of Iowa, L. A. Brown of George Washington University, and A. H. Hersh of Western Reserve University for their many helpful suggestions and criticisms, and especially to Dr. Hersh for suggesting the method of treatment of relative growth.

Summary

Observations as to the number of pre-adult instars have been made on over 200 individually reared female *Daphnia magna* of seven clones. In all cases measurements of the total length during each instar were taken. For well over a hundred individuals measurements of carapace length and height were taken in addition to those of total length.

The number of pre-adult instars varied upward from five. This variation was found in all clones tested.

Growth curves have been constructed for various groups based on the number of pre-adult instars. The inflection in the growth curve of any dimension in any group coincides with the time of sexual maturity.

" Brooks' Law " holds only for those groups which were primiparous during the sixth instar and then only approximately.

Relative growth in the dimensions studied, x and y, may be expressed by the equation

$$y = bx^k.$$

Relative growth changes at sexual maturity, *i.e.*, a change occurs in the values of the constants b and k.

The above equation may perhaps be used to advantage in the study of variation in other Cladocera. By the use of this equation the direction of variation can be given numerical values. Comparisons of differ-

ent races and varieties, both seasonal and geographic, may be readily made on this basis.

BIBLIOGRAPHY

AGAR, W. E., 1930. A Statistical Study of Regeneration in Two Species of Crustacea. *Brit. Jour. Exper. Biol.*, **7**: 349.

BANTA, A. M., 1921. A Convenient Culture Medium for Daphnids. *Science, N. S.*, **53**: 557.

BROOKS, W. K., 1886. Report on the Stomatopoda collected by H. M. S. Challenger during the years 1873–1876. Report of the Scientific Results of the voyage of H. M. S. Challenger during the years 1873–1876. *Zoology*, Vol. 16, Part 45.

CALVERT, P. P., 1929. Different Rates of Growth among Animals with Special Reference to the Odonata. *Proc. Am. Phil. Soc.*, **68**: 227.

DOVER, C., 1931. Effects of Inadequate Feeding on Insect Metamorphosis. *Nature*, London, **128**: 303.

FOWLER, G. H., 1909. Biscayan Plankton Collected during a Cruise of H. M. S. 'Research,' 1900. Part XII. The Ostracoda. *Trans. Linn. Soc.*, London, 2d Ser., Zool., **10**: 219.

GURNEY, R., 1929. Dimorphism and Rate of Growth in Copepoda. *Int. Rev. Hydrob. Hydrogr.*, **21**: 189.

HEBERER, G., 1928. Über eine population von *Daphnia cephalata* King aus Flores. *Zool. Anz.*, Supplementband 3, pp. 70–78.

HERSH, A. H., 1928. Organic Correlation and its Modification in the Bar Series of Drosophila. *Jour. Exper. Zool.*, **50**: 239.

HERSH, A. H., 1931. Facet Number and Genetic Growth Constants in Bar-eyed Stocks of Drosophila. *Jour. Exper. Zool.*, **60**: 213.

HUXLEY, J. S., 1924. Constant Differential Growth-ratios and their Significance. *Nature, London*, **114**: 895.

HUXLEY, J. S., 1927. Further Work on Heterogonic Growth. *Biol. Zentralbl.*, **47**: 151.

RAMMNER, W., 1926. Formanalytische Untersuchungen an Bosminen. *Int. Rev. Hydrob. Hydrogr.*, **15**: 89.

RAMMNER, W., 1927. Die beschreibende und die bildliche Darstellung der Formänderung bei Cladoceren. *Int. Rev. Hydrob. Hydrogr.*, **17**: 115.

RAMMNER, W., 1928. Zur morphogenese und biologie von Chydorus sphæricus und Pleuroxus trigonellus (Ergebnisse aus einzelzuchten). *Zeitschr. Morph. Okol.*, **12**: 283.

RAMMNER, W., 1930*a*. Über die Gültigkeit des Brooksschen Wachstumsgesetzes bei den Cladoceren. *Arch. entw. mech.*, **121**: 111.

RAMMNER, W., 1930*b*. Über milieubedingte Missbildungen bei Daphnia pulex und Daphnia magna. *Int. Rev. Hydrob. Hydrogr.*, **24**: 1.

ROBB, R. C., 1929. On the Nature of Hereditary Size Limitation. II. The growth of parts in relation to the whole. *Brit. Jour. Exper. Biol.*, **6**: 311.

SCHUBERT, A., 1929. Über die (postembryonale) Formentwicklung bei zwei Lokalrassen von Ceriodaphnia reticulata Jurine. *Int. Rev. Hydrob. Hydrogr.*, **22**: 111.

SINGH-PRUTHI, H., 1925*a*. Studies on Insect Metamorphosis. III. Influence of starvation. *Brit. Jour. Exper. Biol.*, **3**: 1.

SINGH-PRUTHI, H., 1925*b*. Moulting of Insects. *Nature*, London, **116**: 938.

SINGH-PRUTHI, H., 1931. Effects of Inadequate Feeding on Insect Metamorphosis. *Nature*, London, **128**: 869.

WERNER, F., 1924. Variationsanalytische Untersuchungen an Chydoriden. Versuch einer quantitativen Morphologie der Cladoceren-Schale. *Zeitschr. Morph. Okol.*, **2**: 58.

WESENBERG-LUND, C., 1926. Contributions to the Biology and Morphology of the Genus Daphnia, with Some Remarks on Heredity. *Kgl. Danske Vidensk. Selsk. Skrifter. naturw. mathem. Afd.*, 8 Raekke, **11**: 89.

WOLTERECK, R., 1925. Notizen zur Biotypenbildung bei Cladoceren. I. Experimentelle Untersuchung der Ceresio-Daphnien. *Int. Rev. Hydrob. Hydrogr.*, **14**: 121.

SOME EFFECTS OF HIGH PRESSURE ON DEVELOPING MARINE FORMS

JOHN W. DRAPER AND DAYTON J. EDWARDS

(From the Department of Physiology, Cornell University Medical College, New York City, and the Marine Biological Laboratory, Woods Hole, Mass.)

The action of hydrostatic pressure, below certain limits, in producing an increase in the contractility of cardiac and skeletal muscle, raises a question of prime interest as to the nature of the effect. Does an increase in the absolute pressure of the environment of a tissue, with the resulting increase in tissue density, give rise to a general stimulation of the fundamental processes in the cells? Several lines of approach have been considered for an answer to this question, but the aim in the present experiments will be to deal only with the influence of pressure on certain of the fundamental processes in the early development of marine eggs.

The experimental observations relate mainly to two features: first, the effect of maintained compression on the rate of development of fertilized eggs, particularly in the early stages; and second, the effects produced by pressure on the rate of the heart in embryos at the stage when pulsations are just beginning and then later when the rhythm is fully established.

The observations were made in an apparatus having essentially the same construction as that previously described (Edwards and Cattell, 1928). The fertilized eggs of *Fundulus* were placed in glass vials filled with sea water and covered with thin rubber membrane to prevent their escape. One lot of the eggs was then placed in the compression chamber, which was also filled with sea water, and another lot, the control sample, was placed in a second chamber similar to the first. In this manner the factors of temperature, respiration and amount of agitation were kept constant, and the experimental sample differed only in having pressure applied for known intervals.

In a few experiments the technic was modified to permit observation of the eggs during the period when they were under the action of pressure. The essential things for this purpose consisted of a heavy glass window mounted in one end of the compression chamber, which made it possible to view with low magnification objects placed immediately beneath it, and a depression slide fixed in position close to the inside surface of the window to contain the eggs. A small mirror backing the slide and a " Pointolite " lamp, which directed a beam of light through the window and against the mirror, permitted fair illumination for a microscopic examination of the eggs in the depression of the slide. All

of the observations on the change of heart rate under pressure were made by using this adaptation of the pressure apparatus.

An observation of general interest arising from these experiments on compression of the developing egg is the extremely slight change that occurs in the egg structures when subjected to comparatively high pressures. The method has permitted observations on the diameter of the egg, the size and state of aggregation of the fat globules, the size and position of the blastodisc, and the size of the finer blood vessels in parts of the embryo. A close study of these different parts of the developing egg, made during and immediately after the onset of pressure of 110 atmospheres, reveals no significant changes. Observations on advanced embryos within the egg, made while pressures of 1500 pounds per square inch were applied, may reveal nothing more than a few quick

TABLE I

The effect of pressure on the cleavage of Fundulus eggs. The time intervals in the fourth column signify the delay in the development of eggs subjected to pressure as compared with those maintained under control conditions. The observations were made at the cleavage stage shown in the fifth column.

Experiment No.	Pressure	Duration of compression	Delay of pressure eggs	Stage of cleavage
	lb.	*min.*	*min.*	
1	1500	140	15	second
2	1700	60	15	first
			13	second
3	1500	108	20	third
4	1950	120	10	third
5	1950	100	15	first
			15	second

movements of the embryo not unlike those shown at indifferent intervals by material of this kind.

In order to test the effects of pressure on the rate of cell division it was necessary to have some standard for comparing the eggs subjected to pressure with those maintained under control conditions. In our initial experiments the completion of the membrane between two daughter cells was taken arbitrarily as an end-point. With this criterion the data contained in Table I show that the eggs subjected to pressure are delayed about fifteen minutes in reaching a given stage of development. The exact time, however, at which a batch of developing eggs reached a given stage in division was often difficult to determine. Additional observations, therefore, were taken (1) by making counts on the control

and the experimental samples to determine the predominant stage at a given time; and (2) by removing samples of 25 or more eggs which were placed in a fixing solution for later examination. The results by these methods of study are set forth in detail in Tables II and III and the evidence they bring forth lends support definitely to the conclusion that compression retards cell division.

The eggs subjected to pressure have been carefully watched for any abnormalities that might occur. A number of monsters have been found of the types showing gross distortions of the body, changes of the cardiovascular system, and tendencies towards anophthalmus. The percentage of abnormal specimens, however, was not large and there was no dominant type of dysmorphism. The abnormalities of the eye were usu-

TABLE II

The effect of pressure on the cleavage of Fundulus eggs. The data contained in the fourth and fifth columns represent simultaneous observations on samples of eggs subjected to pressure (pressure sample) and samples maintained for the control. The observations were made on living material.

Experiment No.	Pressure	Duration of compression	Pressure sample	Control sample
	lb.	*min.*	*division stage*	*division stage*
1	1300	68	1-cell	2-cell
2	1700	112	2-cell	4-cell
3	1650	60	1 and 2-cell	2 and 4-cell
4	1650	30	1-cell	2-cell
5	1575	180	2-cell	4-cell
6	1560	130	1-cell	2-cell
7	1525	140	1-cell	2-cell
8	1300	70	1-cell	2-cell
9	1500	108	2-cell	4-cell
10	1950	120	4-cell	8-cell

ally those showing one located craniad to the other but not exactly in the midline, while the deformities of the cardiovascular system appeared usually as an asymmetrical development of the vessels. The failure of the blood vascular system to develop symmetrically causes the heart to be drawn to one side of the pericardial cavity.

The *Fundulus* embryo is a favorable object for the study of the rate of the heart, since the pulsations of this organ may be observed almost at the time automaticity starts and may be followed until a completely functioning circulatory system is established. As heart automaticity is a fundamental property in development, with a fairly definite time of appearance, the study of the influence of pressure on this phenomenon presented features of unusual interest. The results of 17 experiments

are summarized in Table IV. These data show that a pressure of 1200 pounds produces a reduction in the heart rate within two minutes, amounting to an average decrease of 9.9 per cent from the control rate. The decline in rate continues, however, somewhat more slowly than the initial change, so that at the end of a 10-minute period of compression the average reduction was only 16.6 per cent below the control value. When pressure was applied, the pulsations of the heart showed a definite reduction in the rate within a half minute of the onset. Although in some instances a more or less gradual decline prevails under pressure, the more common type of change appears to be a fairly abrupt slowing.

The action of different amounts of pressure was tested, within the range of 400 to 1200 pounds, in an attempt to determine if critical points

TABLE III

The effect of pressure on the cleavage of Fundulus eggs. The data contained in the fourth and fifth columns represent counts made on samples of eggs subjected to pressure (pressure sample) and samples maintained for the control. The observations were made on fixed material.

Experiment	Pressure	Duration of compression	Pressure sample			Control sample		
			2-cell stage	4-cell stage	8-cell stage	2-cell stage	4-cell stage	8-cell stage
	lb.	*min.*						
1	1500	135	32	1	0	0	35	0
2	1500	135	30	0	0	0	27	0
3	1500	190	20	0	0	0	20	0
4	1500	175	0	28	4	0	0	32
5	1500	140	13	0	0	0	12	0
Total count			96	29	4	0	94	32

exist in the pressure effect. The results support the view that the pressure effect becomes progressively greater with the higher grades of compression on the heart. With pressures ranging from 1200 to 1900 pounds we have been able to suppress the automaticity of the heart to the extent of not being able to observe under low magnification any indication of a contractile response. We do not overlook the fact that localized fine pulsations of a few fibers may have persisted in these preparations, so small indeed as to have been beyond our range of identification, but the essential fact is that automaticity was practically stopped. On release of pressure these hearts which have been held quiescent for several minutes immediately show signs of activity and

eventually recover, thereby confirming our previous observations (Edwards and Cattell, 1928) that the pressure effect is freely reversible in nature. In establishing a pressure standstill of an embryo heart several changes have been observed to occur, such as arrhythmia, partial and complete heart block, and fibrillary motion of the auricles terminating in a cessation of activity in the region of the sinus. With the reëstablishment of activity after release of pressure, sometimes recovery appeared almost simultaneously in sinus, auricle, and ventricle, while in

TABLE IV

Effect of Pressure on the Heart Rate in Fundulus Embryo

Experiment	Age	Control heart rate	Pressure	Heart rate during compression		Heart rate after pressure release		Per cent Δ in heart rate during compression	
				2 min.	10 min.	2 min.	5 min.	2 min.	10 min.
	hr.	*beats per min.*	*lb.*	*beats per min.*	*beats per min.*	*beats per min.*	*beats per min.*		
2	120	68	1200	65	65	60	60	7.3	7.3
3	79	62	1200	58	52	53	—	6.4	16.2
4	96	55	1200	47	43	53	50	9.1	21.9
5a	119	53	1200	48	48	55	54	9.4	9.4
5b	120	55	1200	50	48	—	—	9.1	12.8
6	76	55	1200	50	50	—	—	9.1	9.1
7	121	50	1200	44	46	54	—	12.0	8.0
8	192	62	1200	53	53	62	63	14.5	14.5
9	172	57	1200	54	53	56	—	5.2	7.0
10a	216	65	1200	60	59	67	66	7.7	9.2
10b	217	66	1200	60	—	67	67	9.0	—
11	150	73	1200	67	66	72	—	8.2	9.6
17	74	72	1200	69	63	69	72	4.1	12.6
19	26	60	1200	56	50	53	60	6.6	11.7
20	120	60	1200	52	53	64	60	13.3	11.7
21	144	116	1200	96	100	106	103	17.2	13.8
22	144	112	1200	88	84	99	104	21.4	25.1
Average	....	.....	.....	.....	.....	.....	.....	9.9	16.6

other instances the return followed the reverse order of the disappearance with some degree of arrhythmia preceding the dominance of the normal type.

The remarkable effect of pressure in reducing and abolishing the rhythmicity of the embryo heart raises a question as to the nature of the action. A factor that immediately occurs to one as a possible cause for the restraining action of pressure is a direct excitation of inhibitory nerve fibers supplying the heart. Experiments designed to throw light

on this suggestion were carried out in the following way. Embryos ranging in age from 14 to 20 days were carefully dissected and the heart completely isolated from the surrounding tissue. The preparations were kept immersed in a 40/60 dilution of sea water and tap water to which was added 5 per cent of glucose. These hearts are not easy to handle on account of the very small size, but with care it was possible to mount them on a depression slide and to place them in the compression chamber where their activity could be followed through the window with a low power objective.

The results of these observations confirm in all essentials those obtained with the entire embryos. Moreover, the same type of changes was present, as, for example, the arrhythmia, the different degrees of block in conduction, and the localized areas of rhythmicity. When these results are considered in conjunction with those on whole embryos taken at an early stage before nerve connections are established, they furnish additional support to the view that the depressing action of pressure on rhythmicity is not through an effect on the inhibitory nerve mechanism.

Discussion

In the agent pressure we have an instrument by which the contractility of cardiac and skeletal muscle tissue may be greatly stepped-up, but this action, remarkable as it is for these tissues, appears to be a peculiar effect on the contractile mechanism of these types of muscle. In the present experiments we have evidence that such fundamental biological properties as cell division and automaticity of the embryonic heart become restrained by pressure—a fact that presents equally difficult questions to answer as those given by the augmentation phenomenon in certain types of contractile tissue.

While the types of chemical change underlying the processes of cell division and heart rhythmicity obviously are complex and the factors common to both cannot be set down, yet the fact is not without interest that some forms of chemical reactions are known to be influenced by pressure. Rothmund (1896) found that the acid inversion of cane sugar, a first order catalytic reaction, is decreased in velocity about five per cent when subjected to a pressure of 500 atmospheres, and on theoretical grounds there is reason to conclude, according to Jones (1915), that the velocity of second order reactions is influenced in direct proportion to the pressure.

Attention was called in the early part of this paper to the observation that the cell constituents gave no gross signs of change under pressure.

With albumin, however, an addition of energy to the material produces a tendency to coagulation, as shown by Fermau and Pauli (1915) on irradiating with radium salts, and by Bridgman (1914) with high pressures; therefore it is probable that some alteration takes place in the egg substance even at the comparatively low pressures used in our experiments, but its character is such as to be not easily recognizable. The observations of Heilbrunn (1920, 1921) show in a definite way how great are the changes in viscosity of egg protoplasm during the process of division and they emphasize also the manner in which mitosis may be inhibited by factors that tend to modify the viscosity changes. The pressure effect in retarding cell division may be, therefore, largely one of altering the viscous properties of the cell constituents.

Perhaps the one factor upon which attention first becomes focused in the attempt to account for the decrease in division rate of eggs under pressure is a possible change in the oxygen supply. This feature received thoughtful attention at the outset of our experiments, and we believe that the details of procedure employed provide adequate controls and successfully rule out a change in gas tension as a factor in our results.

Our early observations on the effect of pressure gave evidence to support the view that the rhythmicity of the isolated heart is accelerated when a compression up to 60 atmospheres (882 pounds per square inch) is made to act upon this organ. The procedure in these initial experiments, however, of subjecting the preparation to pressure for only brief intervals and of taking records of the heart cycles immediately following the onset of pressure gave rise to an incomplete conception of the action of this agent. A review of our original tracings and evidence from many additional observations made since show quite conclusively that pressure does not induce an acceleration of the rate of the heart except as a temporary event. The present results, therefore, showing always depression of the rate under pressure seem at first discordant with previous findings, but full consideration of the facts indicates quite clearly that we are dealing with the secondary action of pressure on rhythmicity in contrast to the initial temporary effect that occurred during the first eight to ten cycles in a rhythmic preparation following the onset of compression.

The effect of pressure in slowing the heart rate raises a question concerning the mechanism of this action. While a mechanical factor may have contributed some part in this type of response, it is evident that such an effect does not arise from an increase in the resistance to the

circulation by pressure narrowing the peripheral vessels, since careful observations of the size of certain small vascular channels reveal no detectable alterations in their caliber with the application of pressure. The property of automaticity of the heart, on the other hand, is influenced in a marked degree by changes in the ionic relations of its environment, and the observations of Bogojawlensky and Tammann (1898) on the conductivity-pressure coefficient of some conducting systems gives proof of the changes in the mobility of ions and of the alterations in the ionization of electrolytes by pressure. The precise changes in ionic equilibrium that may have operated in producing a retardation of the heart rate under compression cannot be given from the data at hand, but that a factor of this nature plays a part is strongly suggested by the type of effect that pressure gives in slowing the embryonic heart.

Summary

Hydrostatic pressure of as much as 1900 pounds per square inch applied to living eggs of the *Fundulus* causes no evident changes in the cell constituents.

The rate of cell division of *Fundulus* eggs was slowed by maintaining them under pressures of 1300 to 1900 pounds for periods ranging from 30 minutes to three hours. In the pressure samples a few instances appeared of abnormal forms of development.

The automaticity of the heart in young embryos becomes slowed by pressure and apparently may be abolished if the compression is maintained but returns within a few minutes following release of the pressure. In ten experiments an average decrease of 9.9 per cent in the heart rhythm under pressure was present within an interval of two minutes. The embryonic heart under pressure develops arrhythmia, types of local block, and isolated fibrillary activity.

The action of pressure in slowing the heart rate does not depend on the presence of inhibitory nerves to this organ, since compression produces a slower rhythm of (*a*) hearts from older embryos that have been dissected free of all extrinsic nerve connections; and (*b*) hearts of young embryos that have no intrinsic nerves developed.

The bearing of viscosity and ionic changes produced by pressure are considered in relation to the decrease in the rate of cell division and the automaticity of the embryonic heart.

BIBLIOGRAPHY

BOGOJAWLENSKY, A., AND G. TAMMANN, 1898. *Zeitschr. f. phys. Chem.*, **27**: 457.
BRIDGMAN, P. W., 1914. *Jour. Biol. Chem.*, **19**: 511.
EDWARDS, D. J., AND MCKEEN CATTELL, 1928. *Am. Jour. Physiol.*, **84**: 472.
FERMAU, A., AND W. PAULI, 1915. *Biochem. Zeitschr.*, **70**: 426.
HEILBRUNN, L. V., 1920. *Biol. Bull.*, **39**: 307.
HEILBRUNN, L. V., 1921. *Jour. Exper. Zoöl.*, **34**: 417.
JONES, H. C., 1915. Elements of Physical Chemistry, 4th ed. New York.
ROTHMUND, V., 1896. *Zeitschr. f. phys. Chem.*, **20**: 168.

THE CRUSTACEAN EYE HORMONE AS A VERTEBRATE MELANOPHORE ACTIVATOR

EARLE B. PERKINS AND BENJAMIN KROPP

ZOÖLOGICAL LABORATORY, RUTGERS UNIVERSITY

There is produced in the eye stalks of crustaceans a hormone which, carried in the blood stream, is effective in inducing chromatophore changes. This has been demonstrated repeatedly by Perkins (1928) and Perkins and Snook (1931) for *Palæmonetes,* and by Koller (1928) for *Crangon.* The active material acts in every case as a contractor,—that is, an extract from eye stalks, when introduced into the circulation of blinded or black-adapted shrimps, causes maximal contraction of the red and yellow chromatophores. Koller (1928) also reported the occurrence of the reciprocal phenomenon, namely, expansion of chromatophores of white-adapted shrimps induced by extracts from the rostral region of *Crangon.* Thus far this has failed of experimental confirmation (Perkins and Snook, 1931).

In 1929, Kropp reported the presence in the eyes of black-adapted tadpoles of a substance effective in producing expansion of melanophores in white-adapted *Fundulus* and tadpoles of *Rana clamitans.* In this case the use of extracts from eyes of white-adapted tadpoles gave no results on black-adapted animals. Here, as in the case of the invertebrates, evidence pointed to the presence in the eyes of a substance which, under proper conditions, induced effects on chromatophores.

To test the interspecificity of the chromatophore activator found in the crustacean eye stalk, and thus further to establish its hormone nature, was the object of the experiments here reported. Extracts from the eye stalks of *Palæmonetes vulgaris* were made in 0.7 per cent NaCl solution. Thirty eye stalks were macerated in 5 cc. salt solution, boiled, centrifuged, and the clear extract decanted off and cooled. Tadpoles of *Rana clamitans* were placed in white and black dishes until their skin melanophores were respectively nearly maximally contracted and expanded. To the unaided eye the white-adapted tadpoles appeared light yellow-green, the black-adapted almost black. Each individual then received 0.2 cc. of the extract in the dorsal lymph sinus. White-adapted controls received 0.2 cc. 0.7 per cent NaCl solution. Animals adapted to a black background, which received 0.2 cc. of the eye extract, showed no obvious melanophore movements as a result of the injection, nor did

the controls at any time show marked changes in pigmentation. The animals adapted to a white background, however, did show pronounced effects. Within five minutes of the injection these tadpoles began to darken over most of the dorsal surface, even though they remained on a white background. Maximal darkening was reached after thirteen minutes. The tadpoles at this time showed a very dark band across the

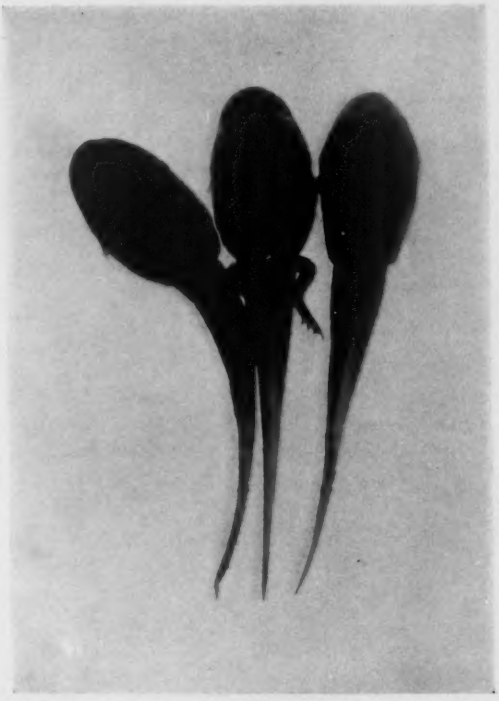


FIG. 1. Left, black-adapted tadpole injected with 0.2 cc. *Palæmonetes* eye extract. Center, white-adapted tadpole injected with 0.2 cc. *Palæmonetes* eye extract: melanophores expanded following injection. Right, white-adapted tadpole injected with 0.2 cc. 0.7 per cent NaCl: no expansion of melanophores.

whole dorsal surface, extending forward to a line connecting the posterior margins of the eyes, and backwards to the anterior root of the tail (Fig. 1). The snout region remained characteristically in the original light condition when the injection was made into the dorsal lymph spaces. We assumed that since the skin between the eyes and over most of the head adheres closely to the cranium, the lack of response of the

head melanophores was due solely to the presence of this mechanical barrier and the consequent inability of the material to come into contact with the skin in this region. This assumption proved correct, for injection of the extract directly under the head skin produced the darkening reaction over the head. The tail did not show the effect, although a suggestion of it could be obtained by injecting the extract directly into

FIG. 2. Left, eye-extract injected white-adapted tadpole. Center, pallor appearing after effect of injecting eye extract had worn off. Sharp line of demarcation between area of punctate melanophores and stellate melanophores of tail clearly shown. Right, normal black-adapted tadpole.

the tail musculature. This is only an apparent anomaly and by no means real, as will be shown below.

Microscopic examination of the skin of the living animal at this time revealed the expected condition—a dense network of expanded melanophores all over the darkened area. Fifteen minutes after the injection the darkening began to disappear until, thirty minutes after maximum darkening, the animal was once more in its original light state, the ef-

fects of the extract apparently having been dissipated. The process of returning pallor did not, however, stop at this point. It continued until the entire dorsal surface of the animal became lighter than it was before the extract was injected. This after-pallor may persist for twenty-four hours or more. It is confined to the dorsum and the root of the tail, the greater mass of the tail being non-reactive and appearing dark in contrast to the pale dorsal surface (Fig. 2). Microscopic examination of the living animal's skin showed, as expected, sharply contracted, punctate dermal melanophores in the trunk, while those of the tail were stellate. The region of demarcation between trunk and tail was strikingly sharp and in different animals was surprisingly similar in contour. The darker area of the tail formed a **V**, pointing cephalad, at a point just posterior to the union of tail and trunk. Laterally the line ran posteriorly and ventrally until near the ventral raphe of the tail, where it continued laterally but anteriorly and was lost in the lightly pigmented skin of the venter. In some animals the line followed superficially the course of the spinal nerves in the tail, while in others there was a more irregular course.

In addition to eye stalks of *Palæmonetes vulgaris* we used those of the blue crab, *Callinectes sapidus*. The active substance was present here also, and the results described above hold true in every detail for tadpoles treated with extracts of eye stalks of *Callinectes*. The only differences observed were that with *Palæmonetes* extracts the onset of the darkening reaction was more rapid, and the degree of maximum darkening attained was somewhat greater.

Since hormones are carried by the blood stream to all parts of the organism, it appears irregular at first sight that the tadpole tail should not respond as did the dorsal surface of the trunk. The tadpole tail has often been an object for experimentation on color change and pigmentary reactions, but results have usually been inconclusive or contradictory (Kropp, 1927). It is indeed not strange that this is so, for the tail of the tadpole, especially in older specimens and when the hind legs have appeared, is in a state of physiological flux. The disorganization of the pigmentary system may be correlated with circulatory changes and the general resorption process of this organ. The fact that the tail is a poorly reacting system is probably due to this physiological instability. In our experiments the reacting substance was carried by lymph and to some extent by the blood stream. For practical reasons we did not inject intravenously at this time. However, we venture the prediction that intravenous injection will not greatly alter the reaction of the tail as described above.

The injected substance has opposite effects in the shrimp and in tad-

poles,—producing contraction of chromatophores in the former, expansion in the latter. This is a further example of a hormone producing different results depending on the reacting system or type of chromatophore concerned.

The chromatophore activator produced by the crustacean eye stalk shows all the characteristics of a true hormone now that we may include its wide interspecificity. This has recently been reported also by Koller and Meyer (1930) and by Meyer (1931), who injected eye extracts and rostral-region extracts of *Crangon* into the fishes *Gobius* and *Pleuronectes,* producing chromatophore movements. With the eye-stalk extract they report the production of pallor in these fishes,—the opposite of the effect we obtained on frog tadpoles.

The fact that an invertebrate hormone is effective on a vertebrate system seems highly significant. As yet very few endocrine processes are known among the invertebrates—the crustacean eye hormone being by far the most spectacular. The attention that has been given to the vertebrate endocrine system has disclosed a variety of humoral effects, but it is by no means certain that this important coördinating mechanism is confined to the vertebrates alone. It is possible that every tissue possesses the ability to affect other tissues by means of substances liberated into the circulating media, not only in the vertebrates but in the invertebrates as well. The occurrence of such a substance in crustaceans which is effective in fishes and amphibians may indicate an evolutionary precursor of the more highly developed vertebrate endocrine system.

CITATIONS

KOLLER, G., 1928. *Zeitschr. vergl. Physiol.,* **8**: 601.
KOLLER, G., AND E. MEYER, 1930. *Biol. Zentralbl.,* **50**: 759.
KROPP, B., 1927. *Jour. Exper. Zool.,* **49**: 289.
KROPP, B., 1929. *Proc. Nat. Acad. Sci.,* **15**: 693.
MEYER, E., 1931. *Zool. Jahrb., Abt. Allg. Zool. u. Physiol.,* **49**: 231.
PERKINS, E. B., 1928. *Jour. Exper. Zool.,* **50**: 71.
PERKINS, E. B., AND THEODORE SNOOK, 1931. *Proc. Nat. Acad. Sci.,* **17**: 282.

"MITOGENETIC RAYS"—A CRITIQUE OF THE YEAST-DETECTOR METHOD

OSCAR W. RICHARDS AND G. WELLFORD TAYLOR[1]

(From the Osborn Zoölogical Laboratory, Yale University, the Physiological Laboratory, Princeton University, and the Marine Biological Laboratory, Woods Hole)

The interest in the theory of mitogenetic radiation has brought forth a considerable literature. Schrieber and Luntz (1931) recently list 49 cases indicating the existence of these rays and 26 cases where negative results were obtained when yeast was used as a detector for the radiation. These rays of short wave length (2000–3400 Å) are supposed to be given off by a wide variety of tissues and to possess the property of accelerating the division of other cells placed in their path. Further, the budding of yeast is generally considered by the proponents of this theory to be especially sensitive to these rays and to be one of the best detectors of this radiation. The literature has been reviewed recently by Hollaender and Schoeffel (1931) and by Taylor and Harvey (1931). Baron (1930) reports mutuoinduction or the acceleration of the budding of yeast by their own radiation.

I

The husbandry of the yeast in many of the published papers on the effects of such radiation is not satisfactory to one familiar with the growth of yeast, and the following minimal essentials are stated to establish criteria for the evaluation of experimental data on the theory of mitogenetic radiation. Violation of these well-established fundamentals of yeast culture accounts for much of the conflicting opinion and inadequate literature on this subject.

First, the yeast used as a detector for this radiation must be a pure strain obtained from an isolated, single cell. Single cell isolation studies by Wallace and Tanner (1928) have shown the amount of variation of different cells from the same pure species of yeast. Second, both the rate of the growth and the yield of yeast should be reported as well as the percentage of cells with buds. An increase in the population, over a period of time, is a better index of proliferation than the percentage

[1] Mr. Taylor expresses his appreciation to the staff of the Woods Hole Laboratory of the U. S. Bureau of Fisheries for facilities placed at his disposal during the summer of 1931 when these experiments were performed.

of buds. Third, the mortality of cells must be determined at the same time. A high mortality would vitiate such experiments and evidence should be produced that there has been no selective killing as normally occurs during the latter part of the growth of a population when the culture medium is not maintained effectively constant (Richards, 1928*a*, *b*, 1932*a*). Fourth, the relative and absolute errors of sampling the populations and of the counting, etc., must be measured and stated. Fifth, the yeast should be maintained at the proper temperature for the species studied and the temperature used stated (*e.g.*, not just as room temperature, Streline, 1929). *Saccharomyces cerevisiæ* shows irregularity of division with elongate cells at 30.0° and injury at higher temperatures.[2] Sixth, the medium should be maintained effectively constant, except for the radiation of the experimental cultures, so that the rate of growth of the yeast otherwise is constant. With many of the culture fluids this limits the length of the experiment with respect to the amount of seeding and the volume of the medium to less than 40 hours unless the medium is changed during the experiment. For a given amount of medium a definitely limited yeast crop will be obtained when the food is exhausted and the waste products accumulate in sufficient concentration to check the growth. With large seedings this crop is obtained in less time than with smaller seedings, even though the rate of growth (percentage and rate of budding) may be the same in both cases. When very small amounts of culture medium, single drops, are used the succession of the known changes which determine the growth of the population[3] would influence and obscure the effects of radiation. This is especially true with experiments on mutuoinduction when the cell densities are proportionally greater.

The proponents of the theory of mitogenetic rays have implied that yeast is unsatisfactory as a detector when it is growing at a constant rate in an effectively constant environment because it is then growing as rapidly as possible. They presume that there is a latent period in cell division which limits the rate of its recurrence. This objection to constant conditions does not affect the experiments to be described because with the same culture conditions the addition of a growth stimulant, such as inosite (Richards, 1932*b*), in a concentration of 1:100,000 will significantly increase the rate of growth. It is also to be remembered that the budding of most of the common species of yeast is different from that of cultures of tissues from multicellular organisms in that the same mother cell may form simultaneously two or more buds. Baron

[2] Borodin (1930) neglects this fact, which probably accounts for much of the variation in his experiments (*cf.* Richards, 1928*c*).

[3] *Cf.* Richards (1932*a*) for measurements and references to the yeast literature.

(1930) states that the initial inoculation must be less than 7,000 per cc. because when a greater concentration is present the cells are saturated with their own radiation and insensitive to radiation from another source. The experiments herein reported had initial concentrations of about 1,200 cells per cubic centimeter.

The observation of the mass of yeast in a hanging drop is not an accurate method, since the cells settle to the bottom of the drop and more cells are present below the center of the drop than at the sides. This error was very obvious in the older type, now generally abandoned, of hæmocytometer made with a center disc surrounded by a circular moat. Even with the use of a planimeter as suggested by Borodin (1931) it is doubtful whether accurate determinations could be made, for the above error of curvature is not avoided and it is exceedingly difficult to estimate the number of cells present from small differences in the apparent optical density of the settled yeast. Counting the cells, the method used in our experiments, not only avoids these special difficulties but also makes possible the estimation of the variation of the measurements, which is important in establishing the accuracy of the method. This is especially important when the method is used to demonstrate a hitherto unknown phenomenon of nature.

II

In the first experiments reported here the yeast was grown in glass or quartz test tubes containing 1.5 to 2.0 cc. of sterile medium and the tubes arranged as shown in Figure 1. The tubes were immersed in a dish containing the bacterial suspension. Sufficient air was drawn through the suspension of bacteria to insure an adequate supply of oxygen. The glass tubes were of ordinary lime glass. The quartz tubes were known to transmit ultra-violet light of the wave length assigned to the mitogenetic rays. This volume of medium was desirable in order that the errors of sampling the yeast population for the counts should be reduced to a minimum. At each time recorded in the tables the cells were shaken to give a uniform suspension and then a small amount of the suspension was transferred to a hæmocytometer and the number of cells present in 5 to 20 unit volumes of 1/250 cu. mm. was counted, using the technique developed by Richards (*1928a, d, 1932a*). The number of buds present was counted and at certain intervals the number of injured or dead cells was determined. No significant differences in mortality were found between control and experimental cultures, and as the mortality during the period of logarithmic growth (rate of growth constant) used in these experiments is slight, this in-

formation is not reported here. The criterion of a bud was arbitrary in that all buds that were seen formed but not yet separated by a wall from the mother cell were counted. Since the senior author made all of the counts of the yeast, the figures are directly comparable. The bacterial suspensions were maintained in a nutrient medium in which they continued to grow over a period of at least eighteen hours. In the longer experiments the bacteria were changed at intervals to insure that a constantly fresh and actively growing suspension was present during the entire course of the experiment. The bacterial cultures were cared for by the junior author. The cultures were maintained in an incubator at $27.8 \pm 0.2°$ C. A special control for each experiment was accomplished by having a glass and a quartz test tube (referred to in the tables

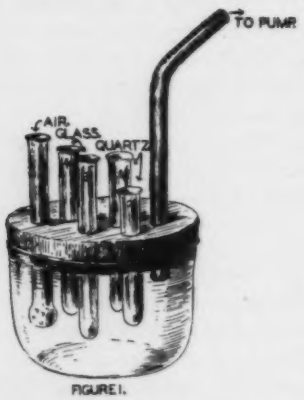


FIG. 1. Arrangement of tubes for Series 109–113. Cotton plugs of tubes, yeast, and bacterial suspensions not shown.

as G_0 and Q_0 respectively) at the same temperature as the other cultures but shielded by glass from the hypothetical source of the mitogenetic rays.

Mitogenetic rays are supposedly effective in accelerating cell division for a distance of several centimeters. The tubes used in these experiments, about one centimeter in diameter, were small enough to insure exposure of all of the cells to any radiation produced by the several sources used. The growth of the yeast in the volumes used was more uniform than usually occurs on agar slants, which generally have been used in the experiments by other investigators. As will be shown later, the actual counts of the population density make certain that there has not been any small increase in the number of cells, that might be missed in counts of the percentage of buds in smears made from the growth on

an agar surface. This is important as these initial small differences would become more divergent as the logarithmic increase continued.

The first two sets of experiments were made with William's medium, a pure strain of *Saccharomyces cerevisiæ* Hansen as the detector, and a

TABLE I

Percentage of Budding and "Induction"

Series No.	Time in hours	PERCENTAGE OF BUDDING						PERCENTAGE OF "INDUCTION" WITH BACTERIA					
		No bacteria (special control)		With bacteria				No bacteria (special control)					
		Quartz tube	Glass tube	Quartz tubes		Glass tubes							
		Q_0	G_0	Q_1	Q_2	G_1	G_1	$\frac{Q_0}{G_0}$	$\frac{Q_1Q_2}{G_1G_1}$*	$\frac{Q_1}{G_2}$	$\frac{Q_2}{G_1}$	$\frac{Q_1}{G_1}$	$\frac{Q_2}{G_1}$
Series 109	2	18	26	20	17	17	27	−31	−16	+18	± 0	−26	− 4
	4	24	28	29	14	20	16	−14	+19	+45	−30	−80	− 1
	6	19	14	25	14	20	20	+36	− 2	+25	−30	+25	−30
	8	14	14	21	23	15	17	± 0	+25	+40	+53	+24	+35
	22	13	12	13	13	12	17	+ 9	−10	+ 8	+ 8	−24	−24
Series 110	2	19	18.5	18.5	17	20	20	+ 5	−16	−11	−22	−11	−22
	4	18.5	20.5	17.5	16	16.5	22	− 1	−12	+ 6	+ 3	−11	−28
	6	16.5	12.5	12.5	15	14	26	+36	−31	−11	+ 7	−51	−44
	9	11.5	13	13.0	16.5	13	16.5	−12	± 0	± 0	+19	−15	± 0
	24	7	10.5	12.5	13.5	14	9	−33	+13	−11	− 4	+39	+50
Series 112	3	28	27	35.5	32	34	28.5	+ 3	± 0	+ 4	− 6	+25	+12
	5	21	26	20.5	16	18	23	−19	−15	+14	−11	−11	−30
	8	18	22	21.5	17	23	21	−18	− 6	− 7	−26	+ 2	−19
	12	25.5	30.5	35.5	37	38.5	33	−16	+ 1	− 8	− 4	+ 8	+12
	24	23.5	24	21.5	20.5	20	25.5	− 2	− 8	+ 8	+ 3	−16	−20
Series 113	3	35	29	30.5	29.5	34	26	+27	± 0	−10	−14	+40	+12
	7	22	27	25	23	25	23	−23	± 0	± 0	− 8	+ 9	± 0
	11	24.5	26.5	22	18.5	23	24.5	− 8	−16	− 4	−20	−10	−25
	24	28.5	28.5	39	42	39.5	42.5	± 0	− 1	−13	+ 6	− 8	− 1

* Calculated from the average percentage of budding with the two quartz tube and the two glass tube populations. Q_1G_2 was calculated from the first quartz tube percentage and from the first glass tube percentage, etc., for all four combinations of the data.

culture of luminous bacteria, from a single cell of *Vibrio phosphorescens*, for the generation of mitogenetic rays. The results are reported in Table I, Series 109 and 110. The percentage of induction, I, was calculated from the formula given by Gurwitsch (1925):

$$I = \frac{Q - G}{G},$$

where Q is the percentage of budding in the quartz tubes and G the percentage of budding in the control or glass tubes. In the special control of a glass and a quartz tube shielded from any rays an apparent positive induction occurred four times out of ten possibilities (Table I, columns Q_0 and G_0), two of which were within the significant range, according to Gurwitsch (1925), of 30–120 per cent even though *no bacteria* were available to generate mitogenetic rays. The rest of the observations were negative or zero. When the percentages of the two quartz and two glass tubes placed in the bacterial suspension were averaged respectively and the averages used to calculate the induction (columns Q_1 Q_2, G_3 G_4), only three positive cases in ten experiments were found, and in none of these was the induction great enough to be significant.

It is further desirable to calculate the induction from the four combinations of the percentages of the first glass control tube population (G_3) and the first and second quartz (Q_1 and Q_2) tubes separately and likewise with the second glass control tube (G_4) and the two quartz tube percentages of budding. Examination of the results tabulated in the last four columns of Table I shows only 15 positive cases of the 40 computed percentages and of these only 7 are of sufficient magnitude to be significant even though all had been exposed to an actively growing culture of bacteria which was present to generate mitogenetic radiation.

Another species of yeast, *Saccharomyces ellipsoideus,* has been suggested as being possibly more sensitive to mitogenetic rays than *S. cerevisiæ,* so we obtained a transfer of culture No. 4116 from the American Type Culture Collection and repeated the above experiment using this species. This time we used malt extract medium, which is a very suitable culture fluid for this species, and the results of this experiment are given as Series 112 in Table I. Distinctly less induction was observed with a preponderance of negative cases. It is noteworthy that one significant positive case of 36 per cent was obtained in the special control without radiation, since this shows the amount of variation occurring with very carefully controlled conditions.

The only explanation for these consistent negative results is the possibility that luminous bacteria, or at least the species here used, do not emit mitogenetic radiation. In order to show that this bacterium is not unique in this respect, the above experiments were repeated using *Phytomonas tumefaciens* (Am. Type Coll. No. 4452), as this species has been reported by other experimenters to be a good generator of mitogenetic rays. The observations are recorded in Table I as Series 113 and only one significant positive case of induction was observed out of eight observations.

During most of the period of the experiments the growth of the yeast is logarithmic. Should there be any acceleration of the multiplication of the yeast due to stimulation by mitogenetic radiation, it should show not only as an increase in the rate of growth but also as an increased yield in the quartz tubes over that obtained in the glass tubes. A slight acceleration, possibly less than would be noticed in the percentages of budding, would be distinctly noticeable by the end of the period of growth. To test this the ratios of the number of cells in the quartz tubes to the number in the glass tubes at the end of each series were calculated. For the control which was shielded from the bacteria the average of the ratios was 0.91, showing that there were slightly more

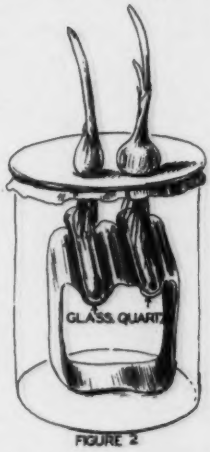


FIG. 2. Arrangement for exposing tubes to radiation from onion roots. When in use, the beaker was partly filled with water to prevent drying of the onion roots.

cells present in the glass than in the quartz tubes. The average of the same ratios for the tubes suspended in the bacterial culture was 0.99. Since twice as many counts were averaged than with the special control, more of the variation is cancelled, which accounts for the index being nearer unity. The maximum yield is less in the quartz tubes than in the glass tubes, which again fails to indicate any stimulation of budding attributable to a mitogenetic radiation.

When the logarithm of the number of cells is plotted against time, the slope of the resulting curve is the relative rate of multiplication of the cells. This graph is linear during all of these experiments except the last observation of five cultures, because the seeding was from ac-

tively growing cultures. These curves were plotted for each tube of the four series and the slope of the curve measured with a tangentmeter (Richards and Roope, 1930). An index was obtained by dividing the tangent of the curve from a quartz tube by that from the corresponding glass tube. The average of these ratios for the control with no bacteria

TABLE II

Percentage of Budding and "Induction"

Series	Time	Budding in Quartz	Budding in Glass	Induction
	hours	*per cent*	*per cent*	*per cent*
117	2	21	24.5	−14
	4	23	28	−18
	6	27.5	28	− 2
	8	33.5	33	+ 2
	11	22.5	26.5	−15
	23	23	23	− 8
118	2	22	33	−33
	4½	28.5	32.5	−13
	8½	35	30.5	+15
	12½	36.5	31.5	+16
	26	27	28	− 4
119	2	37	34.5	+ 7
	5	37	38.5	− 4
	7	35	45	−22
	12.5	35	40	−12.5
	24	37.5	35.5	+ 4
				Summary
				5+ 31%
				11− 69%

Ratios Quartz/Glass

Series	Growth rate	Yield	Detector	Generator
117	1.07	1.11	*S. ellip.*	Onion
118	.96	1.05	*S. cerev.*	Onion
119	1.00	.84	*S. ellip.*	Onion

was 0.97 and for the tubes in the bacterial suspension 0.86. This shows that the rate of growth was slightly greater for the yeast populations in glass tubes, which might be due to some material in the glass dissolving into the medium, which causes a very slight increase of growth. This could not be enough to impair the value of the glass as a control as the differences are but slightly more than the probable error of the differ-

ences, and the sign of the difference was not the same in the several experiments. This is not due to inhibition, because some of the experiments gave greater growth in quartz than in glass. These two additional criteria also indicate that there is no influence passing through quartz but not through glass to stimulate the multiplication of yeast.

Three series of experiments were made using onion roots as the generator of mitogenetic rays and using both species of yeast as de-

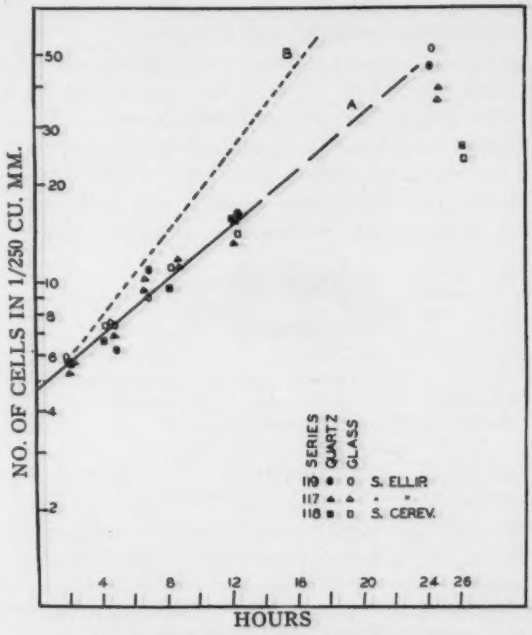


FIG. 3. *A*. The growth curve of Series 117–119. *B*. Hypothetical growth curve that would be obtained with a 10 per cent stimulation of cell multiplication (*cf.* text).

tectors. The arrangement of the experiments is shown in Figure 2. Since the yeast suspension in the tubes covered a considerable area, all of the roots were left on the onion and the positions of the tubes were reversed at each count to insure comparable conditions. The results are given in Table II. The negative cases again predominate and no essential differences are noted in the ratios of the rates of growth or in the yield between the experimental and the control cultures.

The actual counts of the growth of cells are given in Fig. 3, curve *A*. Each point is an average of several counts for a single tube. Because only one tube was used in each experiment, all of the variation is shown in the figure. The growth for the first twelve hours is logarithmic, but the final observation of both Series 117 and 118 was beyond the period of constant growth rate. The effects of a lag period are slight. Careful examination of the points shows no consistent change in the growth that can be interpreted as an acceleration or an acceleration followed by a retardation. The average growth of the onion roots in the moist atmosphere during the experiments was 15 per cent.

The concluding experiments were designed to test whether yeast would stimulate other yeast when the two populations were separated by quartz. The arrangement of the dishes is shown in Figure 4. Two cubic centimeters of the yeast suspension were used for both the generator and detector populations. This amount filled the space in the lower container up to the quartz partition and is about the smallest amount that allows counts without considerable errors in sampling.

FIG. 4. Cross-section showing arrangement of a quartz dish within a Petri dish. The detector yeast was placed at *A* and the generator yeast at *B*.

The observations are given in Table III. Two duplicate experiments were made in each case. The induction percentages for the averages of both duplicates give an equal number of positive and negative cases in none of which are the differences great enough to be significant. When all combinations of the experiments are calculated, there is again an excess of negative cases. The average figures suggest that there may be some increased budding in the quartz dishes at two to four hours after seeding. These differences are not of sufficient magnitude to be significant and in the case of Series 119, the positive deviation is due to variation in only one of the two populations.

III

There is noticed a considerable variation between the different cultures even though conditions were made as nearly uniform as possible. The extent of this natural variation was measured in several cases, two of which are given in Table IV showing the percentages of buds counted and the probable error of the percentages. This was obtained by keep-

ing a separate record of the counts in each of the 20 unit volumes counted and the usual statistical formula applied. With divergent percentages (Case 1) the induction varies from 2 per cent to 35 per cent for values within the range (mean ± its probable error) wherein the

TABLE III

Percentage of "Induction"

Series	Time	Percentage of induction Av. Glass Av. Quartz	Q_1G_1	Q_1G_2	Q_2G_1	Q_2G_2
	hrs.					
117a	2	+ 2	± 0	+ 4	± 0	+14
	4	+27	+39	+45	+ 9	+14
	6	± 0	−13	+11	− 6	+20
	8	−26	−26	−26	−26	−26
	11	−17	−11	−14	−16	−19
	23	−20	−15	−19	−19	−27
118a	2	−12	−21	−11	−13	− 2
	4	+28	+25	+21	+27	+33
	8	−13	− 6	−17	− 7	−18
	12	+ 3	−19	−14	+19	+28
	26	+11	+ 7	+ 7	+15	+15
119a	2	− 4	+27	− 1	− 4	− 8
	5	+14	−21	−10	− 4	+ 8
	7	−12	− 1	− 7	− 9	−14
	12.5	+12	+15	+30	−11	+ 2
	24	− 2	−11	− 7	+ 4	+ 9
		Summary 8+ 50% 8− 50%		Summary 25+ 38% 2±0 4% 37− 58%		

Initial concentration of yeast (cells in 1/250 mm.³)

Series	Generator	Detector
117a	70.8 *S. ellipsoideus*	11.0 *S. ellipsoideus*
118a	47.1 *S. cerevisiae*	6.4 *S. cerevisiae*
119a	39.0 *S. cerevisiae*	5.1 *S. ellipsoideus*

occurrence of another observation is expected to be equally probable. This result is important, as percentages of induction greater than 30 per cent are significant according to Gurwitsch (1925). Where the values approach each other more closely (Case 2), the induction calculated varies from — 2 per cent to + 33 per cent; or from a negative

result (less budding in quartz than in glass) to a significantly positive result. This clearly shows that the index of induction obtained from this formula is invalidated by the *normal variation* of the biological material and is, therefore, *not a satisfactory criterion* for establishing the existence of mitogenetic radiation.

Gurwitsch (1931) has recently indicated that continued radiation may produce an inhibitory effect on cell multiplication. The above experiments do not support this hypothesis because in them the direction of variation is independent of time. Further, it would be improbable that the inhibition would be such as to give, in all cases, the same or

TABLE IV

Effects of Variation on "Induction" *

	PERCENTAGE OF BUDS COUNTED		PERCENTAGE OF "INDUCTION"
	Quartz tubes	Glass tubes	
Case 1	30.5 ± 2.1	26.0 ± 1.8	
	30.5	26.0	+17
	30.5 + 2.1 = 32.6	26 − 1.8 = 24.2	+35
	30.5 − 2.1 = 28.4	26 + 1.8 = 27.8	+ 2
	32.6	27.8	+17
	28.4	24.2	+17
Case 2	35.9 ± 2.4	31.5 ± 2.7	
	35.9	31.5	+14
	38.3	28.8	+33
	33.5	34.2	− 2
	38.3	34.2	+12
	33.5	28.8	+16

* In each case the mean and its probable error are given. The "induction" is calculated with the Gurwitsch formula (*cf.* text) from the mean values and for the combinations of the mean values both plus and minus the probable errors of the respective means.

slightly less variation than the amount of control growth when the concentrations of the generator and detector populations varied over the limits used in our experiments (*cf.* Table III).

Baron (1930) reports mutuoinduction in yeast populations when he used hanging drops with different densities of cells and rounded up or spread out in form. He starts with cells having no buds and reports the percentage of cells which still have no buds at each observation. The difference in the percentages indicated increased budding when the cells were in close proximity, which increase passed through a maximum and then decreased. A few negative cases were found and explained

by having too densely seeded original populations. Baron attributes the shortening of the lag period to premature stimulation of budding by the radiation of the cells on themselves, or by the radiation of other cells, depending on the arrangement of the experiments.

Three other explanations are possible besides the one that he gives. Eijkman (1912) has shown that the order of spore germination is logarithmic. The recovery from previously unfavorable environmental conditions which stopped budding is also probably logarithmic, so that the numbers would pass through a maximum as the logarithmic order was decreased by the increasingly unfavorable conditions of the small, single drop environments. Another possibility is that the presence of more cells may reduce the lag period by some chemical effect of the aggregation, of the nature discussed by Allee (1930). This is less likely, since Peskett (1927) was unable to demonstrate any allelocatalytic effects on the growth of yeast. Possible effects of a chemical conditioning of the medium should be tested for when large concentrations of cells are present, before a decreased lag period can be attributed to the effect of autoradiation.

Baron did not report any counts of the cells present so a more complete analysis of this experiment is not possible, nor is it clear that he worked with homogeneous material derived from a single cell isolation. It is possible that his denser suspensions used up the food in the drops more rapidly and that and the resulting increase of toxic waste products brought about the diminution of budding which he observed. Slator (1921) has stated that a considerable concentration of cells will fail sometimes to come out of the lag period and increase their number. A combination of these effects could give Baron's results, and it is believed his observations can be accepted only when he measures the changes in the environments to demonstrate that the environmental changes have no effect that might be attributed to radiation, or better, maintains the environments effectively constant except for the radiation of the experimental cultures. The changes in the environment as cells grow when the medium is not renewed have been measured for larger environments, but not as yet for the smaller environments. When the environment is restricted to a single drop, these changes must regulate the growth of the yeast more effectively than with larger environments. It is probable that failure to avoid the effects of such changes accounts for the difficulties that have prevented direct comparison of experimental findings with hanging drop and agar block cultures.

Clark (1922) varied the seeding from five cells to eight million cells per cubic centimeter with a strain of *S. cerevisiæ* different from that used in our experiments and found no significant differences in the rate

of growth of his populations that could be attributed to a mutuoinduction of the cells upon each other. Richards (1932*a*) found that the rate of growth, with the same strain of yeast, was the same when the initial seeding was varied from fifteen thousand to one and one-half million yeast cells per cubic centimeter of medium.

Streline (1929) finds an initial stimulation of growth due to mitogenetic radiation which rapidly disappears. He emphasizes the need for counts of the number of cells present and for liquid culture medium. Streline uses *Nadsonia fulvescens*, which has a different form of budding from the yeast reported in this paper, and in reporting his experiments counts two small buds as one cell. This prevents further analysis of his data, and without knowledge of the mortality of his cells it is not possible to analyze the interesting disappearance of the stimulation. He, too, reports some negative cases.

IV

When there is no killing of the cells in the population, any stimulation of cell proliferation would give greater differences between the size of the control and the experimental populations. For instance, had there been a 10 per cent increase of cells in the experiments reported in Fig. 3, curve *A*, during the first two hours that had continued, the increase that would have occurred is shown by the broken line, Fig. 3, curve *B*. Had there been an initial stimulation that had not continued, then the growth curve would have been a line above and parallel to the growth curve of the control. The figure demonstrates that no such stimulation occurred in our cultures.

In our experiments the environment was maintained effectively constant, except for the possible radiation of the experimental cultures, and the actual variation in both the number of cells present and the number of buds and in the death rate was measured. From these data the rate of growth and the percentage of budding were calculated.

These carefully controlled experiments, using *S. cerevisiæ* and *ellipsoideus* for detectors, and onion root, two species of bacteria—*V. phosphorescens* and *P. tumefaciens*—and the two above-mentioned yeast as generators, show no stimulation of the multiplication of yeast by mitogenetic radiation that might pass through quartz but not through glass.

It is not possible to compare our experiments closely with those obtained by other investigators because the latter have not given their complete data. Only when the complete evidence, as has been indicated in Sections I and III of this paper, is given together with a definite acceleration of the multiplication of yeast by mitogenetic radiation, can a

theory of mitogenetic radiation be established. The formula for the percentage of induction proposed by Gurwitsch is inadequate and unsatisfactory because it too greatly exaggerates the normal variation of the growth of yeast.[4]

SUMMARY

Yeasts (*Saccharomyces cerevisiæ* and *ellipsoideus*) grown in a liquid medium which was maintained effectively constant in quartz and in glass containers, were exposed to supposedly potent sources of mitogenetic radiation: bacteria (*Vibrio phosphorescens* and *Phytomonas tumefaciens*), onion roots, and the two above-mentioned species of yeast. In 58 per cent of the experiments, negative results were obtained, while in 42 per cent there was a slight positive variation which did not exceed normal control variations. No effects of a mitogenetic radiation could be detected in the exposed yeast. In all cases the percentage range of variation of the experimental cultures was within the range of normal variation.

It is further shown that the formula used by the Gurwitsch school in calculating the induction effect is unsatisfactory because it exaggerates the variations found in the normal cultures until they appear as induction or inhibition effects.

The necessity of an effectively constant environment which does not restrict the potentially unlimited growth of the yeast is stressed. A constant environment avoids the difficulties of measurement and evaluation of the unfavorable effects of changing environments in experiments designed to test the effect of mitogenetic radiation or other stimulants of yeast growth.

Thus certain conditions and criteria are established which, it is believed, the proponents of the theory of mitogenetic radiation cannot legitimately disregard in their attempt to establish the existence of such a radiation when yeast cultures are used as detectors.

BIBLIOGRAPHY

ALLEE, W. C., 1930. Animal Aggregations. Chicago.

BARON, M., 1930. Analyse der mitogenetischen Induktion und deren Bedeutung in der Biologie der Hefe. *Planta*, **10**: 28.

BORODIN, D. N., 1930. Energy Emanation During Cell Division Processes (M-rays). *Plant Physiol.*, **5**: 119.

BORODIN, D. N., 1931. Biological Spectrum and M-rays. *Collecting Net*, **6**: 274.

CLARK, N. A., 1922. The Rate of Formation and the Yield of Yeast in Wort. *Jour. Phys. Chem.*, **26**: 42.

EIKMAN, C., 1912–13. Verslagen k. Akad. Wetensch., Amsterdam, *Wis-en Natuurk.*, **21**: 507. (From Rahn.)

[4] If the conclusions of Rahn (1932) are correct, this normal variation never can be avoided completely.

GURWITSCH, A., 1925. Methodik der mitogenetischen Strahlenforschung. *Abderhalden's Handb.*, Abt. 5, T. 2, H. 13: 1401.

GURWITSCH, A., 1931. Die fundamentalen Gesetze der mitogenetischen Erregung. *Arch. Exper. Zellforsch.*, **11**: 1.

HOLLAENDER, A., AND E. SCHOEFFEL, 1931. Mitogenetic Rays. *Quart. Rev. Biol.*, **6**: 215.

PESKETT, G. L., 1927. Studies on the Growth of Yeast. III. *Biochem. Jour.*, **21**: 104.

RAHN, O., 1932. A Chemical Explanation of the Variability of the Growth Rate. *Jour. Gen. Physiol.*, **15**: 257.

RICHARDS, O. W., 1928*a*. Potentially Unlimited Multiplication of Yeast with Constant Environment, and the Limiting of Growth by Changing Environment. *Jour. Gen. Physiol.*, **11**: 525.

RICHARDS, O. W., 1928*b*. Changes in Sizes of Yeast Cells during Multiplication. *Bot. Gaz.*, **86**: 93.

RICHARDS, O. W., 1928*c*. The Rate of the Multiplication of Yeast at Different Temperatures. *Jour. Phys. Chem.*, **32**: 1865.

RICHARDS, O. W., 1928*d*. The Growth of the Yeast Saccharomyces cerevisiæ. I. The growth curve, its mathematical analysis, and the effect of temperature on the yeast growth. *Ann. Bot.*, **42**: 271.

RICHARDS, O. W., 1932*a*. The Second Cycle and Subsequent Growth of a Population of Yeast. *Arch. f. Protist.* In press.

RICHARDS, O. W., 1932*b*. The Increased Growth of a Population of Yeast with Inosite. *Proc. Soc. Exper. Biol. Med.*, **29**: 627.

RICHARDS, O. W., AND P. M. ROOPE, 1930. A Tangent Meter for Graphical Differentiation. *Sci.*, **71**: 290.

SCHRIEBER, H., AND A. LUNTZ, 1931. Mitogenetische Strahlung. *Tabulae Biol. Period.*, **1**: 285.

SLATOR, A., 1921. Yeast Crops and the Factors which Determine Them. *Jour. Chem. Soc.*, **119**: 115.

STRELINE, G. S., 1929. [In Russian.] *Věstn. Rentgenol.*, **7**: 191.

TAYLOR, G. W., AND E. N. HARVEY, 1931. The Theory of Mitogenetic Radiation. *Biol. Bull.*, **61**: 280.

WALLACE, G. I., AND F. W. TANNER, 1928. Studies on the "Bios Question." *Centrlbl. f. Bakt. u. s. w.*, **76**: 1.

RATE OF REGENERATION OF PARTLY HISTOLYZED ANURAN TAIL SKIN [1]

H. J. CLAUSEN

DEPARTMENT OF BIOLOGY, UNIVERSITY COLLEGE, NEW YORK UNIVERSITY

INTRODUCTION

Recent studies on anuran integument and muscle transplantations (Helff, 1926; Lindeman, 1929*a*; Helff and Clausen, 1929; and Clausen, 1930) all suggest the probability that the causal histolytic influence of the various tissues of the tail may be due to specific substances in the blood stream or a general lowering of the pH of the blood during metamorphosis. It has been suggested (Lindeman, 1929*b*) that this influence is essential, not only as an initiatory agent, but must also be present continuously during tail resorption. This was demonstrated by the transplantation of histolyzing anuran tail-skin to normal individuals, resulting in the normal reconstitution of the graft in question. The various intermediate regenerative stages were not described, however. Part of the present paper is devoted, therefore, to a description of the normal histological regenerative processes, which occur in tail integument following partial histolysis.

In a previous paper (Clausen, 1930) a susceptibility gradient to histolysis was demonstrated for various regions of tail-skin. In this connection it was clearly shown that integument derived from anterior regions of the tail undergoes a more rapid rate of histolysis than is true of integument derived from more posterior regions. It was, consequently, thought of interest to determine whether or not any differences existed between the rates of regeneration of tail-skin derived from various anterior-posterior levels of the tail. Work on amphibian tail regeneration by several investigators seems to have given rise to rather diverse results and interpretations. Morgan (1906), working with the salamander *Diemyctylus viridescens,* and Ellis (1908, 1909), on the anuran *Rana clamitans,* seem to agree in a general way that the rate of regeneration of new portions of the tail is directly proportional to the distance the tail is cut off from the tip. In other words, the more distal the cut, the slower the regeneration. Conversely, Comes (1928), working with the anuran *Discoglossus pictus,* states that regeneration of the

[1] This paper, together with Parts I and II of this series of studies, was submitted to the Graduate School of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, April 1, 1932.

tail fin diminishes anteriorly and with the age of the animal. Speidel (1929), however, using several types of anurans and salamanders, reports no differences in the rate of resorptive or regenerative processes of the tail fin at anterior, middle, or posterior levels following thyroid treatment. Since the investigations of Morgan and Ellis on tail regeneration concern regeneration of all tissues of the tail, and the work of Comes and Speidel concerns the rate of tail fin regeneration *in situ*, it seemed of interest to the writer to inquire into the possible regenerative rate differences of only one type of tail tissue as might be demonstrated by transplantation to foreign regions. By so doing, it was thought possible to demonstrate whether a difference in regenerative rate was due to the association of the tissue with a definite level of the tail and adjacent tissues or whether the particular tissue in question possesses a specific rate of regeneration typical of integument of that region. As far as the writer is aware, the present paper constitutes the first attempt to explain the above points, especially as regards the rate of regeneration of histolyzing tail-skin taken from various levels of the tail.

The general methods of attack which were followed in the present investigation were briefly as follows: The integument from four levels (anterior-posterior) of the tail of normal larvæ was transplanted, autoplastically, to the back. Following this procedure, artificial metamorphosis was induced and the engrafted integument removed at certain stages of histolysis and transplanted, homeoplastically, to the back or tail of normal individuals. Subsequent macroscopic and microscopic observations were made of the various transplants at certain definite intervals as regeneration progressed. The following series of transplantations, which will be described in detail in the respective sections, were performed:

Series 1. Autoplastic skin transplantations from four regions of the tail to the back. (One hundred and twenty cases of four transplants each.)

Series 2. Skin transplants (previously transplanted in Series 1 and in various stages of histolysis) transplanted homeoplastically to the tail or back. (Two hundred and twenty-five cases of two transplants each.)

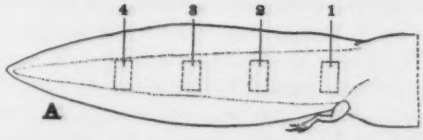
Series 3. Homeoplastic skin transplantations of four regions of the tail of normal larvæ to the tail or back of normal larvæ. (Thirty-two cases, control series.)

This investigation was undertaken at the suggestion of Dr. O. M. Helff, to whom the writer wishes to express his appreciation for his helpful suggestions, kindly advice, and criticism during the course of the work.

Material and Methods

The stock used for all operations was *Rana pipiens* larvæ obtained from ponds in the vicinity of Cold Spring Harbor, Long Island, during the months of June and July, 1931. The animals were taken to the laboratory and placed in a large tank supplied with fresh running water. It was found advisable to let the stock remain in the large tank from four to five days under laboratory conditions before selecting individuals to be used for experimental purposes. By doing this, only those individuals which survived the new environmental conditions would be selected. These animals ranged from 70 to 90 mm. in body length with hind limbs between 3 and 12 mm. in length. All experimental individuals were normal larvæ in all respects and remained as such until induction of artificial metamorphosis following the autoplastic operations. All operated animals were kept in individual aquaria and maintained under constant laboratory conditions.

With the exception of a small number, it was found necessary to anæsthetize all animals used for transplantation purposes. The animals were anæsthetized in a 0.05 per cent aqueous solution of chloretone for



five to ten minutes, depending on individual differences and the temperature of the solution. These anæsthetized animals remained inactive sufficiently long to carry out operative procedures and insure adhesion of the transplants and their cut edges.

In the first series of experiments, autoplastic transplantations were made consisting of the removal of tail-skin from regions 1 and 3 or from regions 2 and 4 (text figure *A*). These portions of integument were then carefully shaped into rectangles measuring approximately four millimeters in width and seven millimeters in length. Two similar-sized and -shaped rectangular portions of integument from the medial region of the back were next removed and the tail-skin transplants from regions 1 and 3 or 2 and 4 were then transplanted to the denuded areas of the back. As regards the orientation of the grafts, the linear sequence of transplants number 1 and 3 or 2 and 4 were alternated in 50 per cent of the cases. In other words, graft number 1 or 2 was grafted to the more anterior wound area, leaving graft number 3 or 4 to be transplanted to the remaining posterior wound area; while in other cases

graft number 3 or 4 was placed more anteriorly. After the above transplantations were made, the animal was placed in a Petri dish containing a small amount of water. This procedure allowed the grafted areas to be exposed to the air and hastened the adherence of the cut edges. Following this, the animals were placed in individual aquaria containing water which was kept at a temperature ranging from 18° to 20° C. After five days, the time allowed for sufficient healing of the graft to take place, precocious metamorphosis was induced by the feeding of desiccated thyroid.

During larval transformation, daily macroscopical observations were made of the above individual transplants to note the reduction in area of the grafts. When the grafted integument had been reduced in area approximately eighteen per cent, a number of grafts originally derived from tail regions 1, 2, 3, and 4 were removed and transplanted, homeoplastically, to normal larvæ. The same procedure was carried out for a like number of grafts when the reduction in area had reached approximately 50 and 80 per cent. In making the homeoplastic transplantations, the histolyzing graft was removed and placed on previously denuded areas of the back or lateral regions of the tail of normal larvæ. Following these transplantations, the normal larvæ were again placed in individual aquaria and kept as such under constant laboratory conditions. At intervals of 8, 11, 14, 17, 20, 23, and 26 days, following the homeoplastic operations, representative individuals possessing the transplanted integument of each of the four tail regions which had previously been reduced in area by 18, 50, or 80 per cent, were removed from their individual aquaria and preserved *in toto* in Bouin's Picro-Formol fluid for subsequent sectioning and histological study of the grafts.

A second series of homeoplastic transplantations was also made. The technique consisted of removing rectangular portions of tail-skin from regions 1, 2, 3, and 4 of normal larvæ. These normal skin transplants were then placed on denuded areas of the back or tail of other normal larvæ. Individuals of this series were also preserved for histological study of the transplants at 8, 11, 14, 17, 20, 23, and 26-day intervals following transplantation. This series was designed as a control to make certain that histolysis does not occur in larval skin following homeoplastic transplantation to normal larvæ.

Results

The Process of Degeneration and Regeneration in Tail Integument

1. *The normal integument.*—The normal tail integument in the particular species studied (*Rana pipiens*) presents a macroscopical picture differing in appearance from integument of body regions. It appears

much more translucent and is also decidedly more delicate in texture, being injured and torn more readily than integument of the side or back. It is also attached more rigidly to the underlying musculature as compared with back-skin. The pigmented areas are regularly distributed over the entire tail region and appear to be less intense in depth of shade as compared with similar areas of the back.

As to histological structure, tail-skin is composed of an epidermis, a corium, and a layer of subcutaneous connective tissue. The epidermis is composed of several layers of cells, the outermost of which consists of a single layer of small, flattened cells forming the cuticle. The more basal cells of the epidermis are for the most part cuboidal in shape with round or oval-shaped nuclei. The epidermal cells basal to the cuticle are usually arranged into two or three irregular layers. The corium, which is separable into two definite layers in most regions of the body, differs markedly in this respect in tail integument. The outer, comparatively loose layer (stratum spongiosum), so typical of side and back integument, is almost obliterated in tail integument. This layer is represented, however, by a thin layer of pigment cells lying directly beneath the epidermis. The lower layer of the corium (stratum compactum) is composed of dense connective tissue, the fibers running in a wave-like course parallel to the surface. The characteristic much convoluted appearance, typical of this layer in body regions, is absent in tail integument. This layer is, moreover, much thinner as compared with the condition found in back integument. These corionic modifications, typical of tail-skin, are responsible for the latter's relative thinness and more delicate structure as compared with integument of body regions. The subcutaneous connective tissue forms a loose layer underneath the stratum compactum. This layer is quite vascular, containing large numbers of blood cells, chiefly lymphocytes. It is also considerably thinner than the subcutaneous connective tissue layer beneath back integument.

2. *The degenerative process.*—The same histolytic characteristics are exhibited for all four anterior-posterior transplants of tail integument when the degree of resorption, as measured by reduction in surface area, is the same. Therefore, in the explanations to follow, the process of degeneration will be described only as observed in tail-skin from region number 1 (text figure *A*). When the transplant had been reduced in surface area 18 per cent, no apparent macroscopical differences as compared to normal integument of the tail could be noted. The pigmentation remained normal throughout this period of resorption (Fig. 3). However, when the transplants had been reduced 50 and 80 per cent, respectively, a decided change in depth of shade could be noted.

In other words, the greater the degree of histological disintegration, the darker in color the transplant became (Figs. 1 and 2). This increase in depth of coloration proceeded from the margins of the transplant toward the center as histolysis progressed.

In examining histological sections of the tail-skin transplants at various stages of area reduction, the first signs of histolysis are concerned with the structure of the stratum compactum. When the transplant had been reduced in surface area 18 per cent, the stratum compactum had lost its wavy appearance; the fibers becoming more or less dissociated so that a very disorganized picture is presented (Fig. 5). With this dissociation, lymphocytes make their appearance, apparently migrating from the subcutaneous tissue into the compactum layer. These lymphocytes are particularly abundant at the edges of the transplant, where fusion has occurred with the surrounding integument of the back. At this stage of transformation the epidermis appears to be thickening, especially near the central region of the graft.

When the engrafted integument had undergone resorptive changes amounting to 50 per cent of its original surface area, the stratum compactum was entirely obliterated leaving only scattered pigment cells, lymphocytes, subcutaneous connective tissue, and the epidermal layers. An interesting histological feature of the graft in this stage of histolysis is the increased thickness of the epidermal portion, which appears to be several layers thicker at this time (Fig. 6). The basal layer of this

PLATE I

Explanation of Figures

FIGS. 1–5. *BI*, adjacent back integument; *P*, pigment masses; *TST*, tail-skin transplant; *E*, epidermis; *SS*, stratum spongiosum; *SC*, stratum compactum; *CT*, subcutaneous connective tissue; *L*, lymphocytes; *TT*, boundaries of transplant; *BV*, blood vessel.

FIG. 1. Macroscopic appearance of tail-skin graft histolyzed 50 per cent its original surface area.

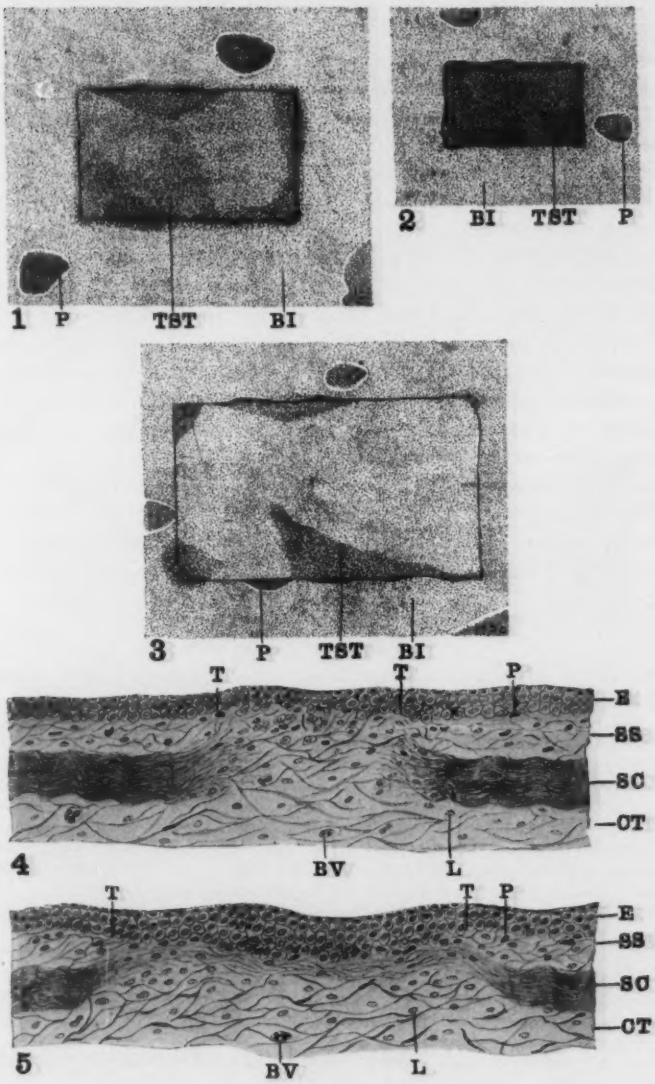
FIG. 2. Macroscopic appearance of tail-skin transplant histolyzed 80 per cent its original surface area.

FIG. 3. Macroscopical picture of normal tail-skin transplant as it appears five days following autoplastic transplantation.

FIG. 4. Histological section through autoplastic tail-skin transplant and adjacent back-skin at a time when the graft had histolyzed 80 per cent (surface area reduction). The stratum compactum is obliterated. The epidermal layer is beginning to dissociate and lymphocytes are migrating into this particular region. There is also a slight condensation of pigment cells basal to the epidermal portion of the transplant.

FIG. 5. Histological section through autoplastic tail-skin graft and adjacent back integument when the transplant had histolyzed 18 per cent. The stratum compactum has lost its wavy appearance and is becoming more or less dissociated. Subsequent lymphocytic invasion is shown following this dissociation. The epidermal portion of the graft appears normal at this time.

PLATE I



thickened epidermis contains cells which have hypertrophied and assumed a more columnar shape. In sections of tail-skin grafts, where the original surface area had been reduced approximately 80 per cent (Fig. 4), the previously thickened epidermis, as illustrated in Figure 6, is now very much thinner. Sections show a separation and basal migration of groups of these epidermal cells. With this epidermal dissimilation there is a gradual invasion inward of all layers of the surrounding back integument. This invasion probably places the graft under a certain degree of peripheral pressure. The lymphocytic cells are now found scattered throughout all the various layers of the graft, including the epidermis. The cuticle is still intact at this stage and apparently is the last layer to exhibit a histolytic change.

The above histological and macroscopical findings show that the integumentary grafts exhibit histolytic processes which are identical with those typical of histolyzing tail integument, *in situ*, during normal metamorphic tail atrophy. In this connection, it may be stated that a more complete study of the histological processes which take place during histolysis was not attempted; only those sections taken at definite periods, when surface area had been reduced 18, 50, and 80 per cent, having been studied. It may be stated here, however, that the microscopical pictures of tail-skin histolysis as thus observed compare favorably with those of Helff (1926) and of Lindeman (1929*a*).

PLATE II

Explanation of Figures

FIGS. 6–10. Histological sections through back integument including homeoplastic tail-skin transplants originally derived from the most anterior regions of the tail. The sketches illustrate successive steps in the histological regeneration of the transplants which had previously undergone partial histolysis following autoplastic transplantation. *E*, epidermis; *SS*, stratum spongiosum; *SC*, stratum compactum; *CT*, subcutaneous connective tissue; *P*, pigment masses; *TT*, boundaries of the transplant; *L*, lymphocytes; *BV*, blood vessel.

FIG. 6. Section through autoplastic tail-skin graft and adjacent back integument showing condition of transplant when histolyzed 50 per cent (surface area reduction). The stratum compactum is nearly obliterated. The epidermis is increased in thickness with its more basal cells somewhat hypertrophied.

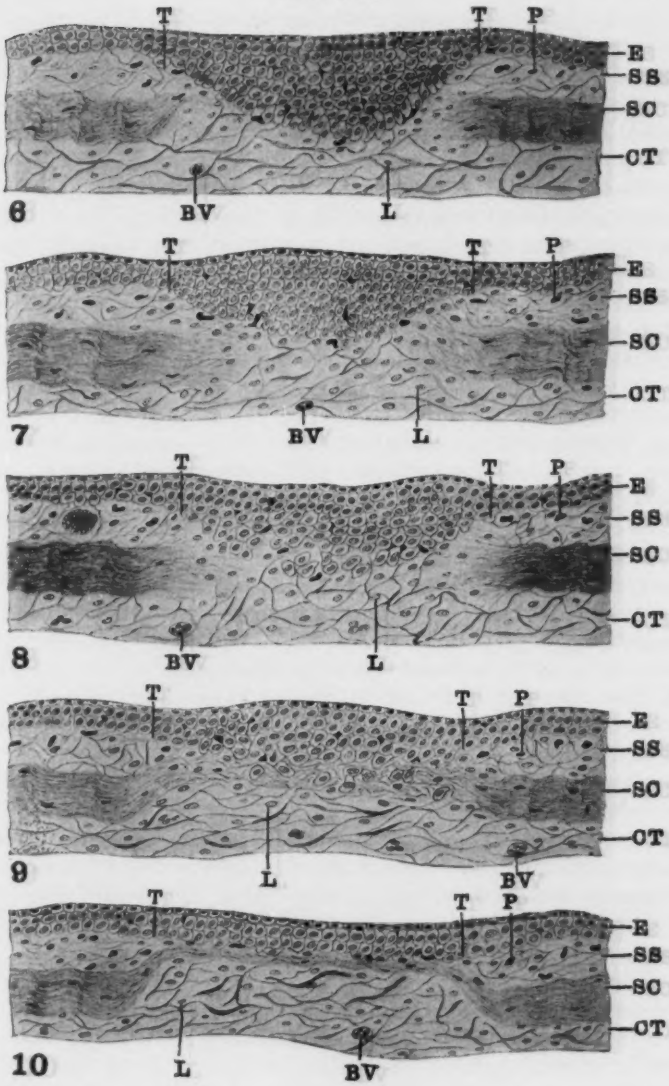
FIG. 7. Eight days following homeoplastic transplantation. The first signs of regeneration as shown by a condensation of the epidermis.

FIG. 8. Eleven days following homeoplastic transplantation. Basal cells of the epidermis breaking off and separating from the main portion of that layer.

FIG. 9. Fourteen days following homeoplastic transplantation. Epidermis still dissociating in the basal layers. Evidence of a stratum compactum layer, its fibers being more or less loosely associated.

FIG. 10. Seventeen days following homeoplastic transplantation. Histologically, the transplant appears as normal tail integument.

PLATE II



3. *The regenerative process.*—Tail-skin transplants reduced in surface area 50 per cent were used as typical forms in the following description of the regeneration of histolyzing tail-skin. The histological pictures (Figs. 6–10) illustrate the process of regeneration from the time of transplantation (homeoplastic) of the histolyzing graft to normal larvæ, to the time when the graft in question had again assumed a normal histological picture. Sections showing the intermediate periods were taken at three-day intervals following the five-day period allowed for healing of the homeoplastic transplants.

In macroscopical appearance (Fig. 1) the graft, prior to the homeoplastic transplantation, appears much darker in color than the normal integument of the tail region from which it was originally derived. This darkened coloration is especially noted as being more intense near the margins of the graft. The depth of coloration remained as such for approximately eight days following homeoplastic transplantation. From this time on the transplant began to assume a lighter color; the margins of the graft, however, always appearing darker as compared with the more central regions. Normal coloration of the integumentary transplant, however, was not attained until approximately fourteen days following the homeoplastic transplantation.

Several transplants from tail region 1 were sectioned at the time homeoplastic transplantations were usually made. Sections were also made of transplants after 8, 11, 14, 17, 20, 23, and 26 days following homeoplastic transplantation to normal larvæ. Histological examinations show that subsequent changes in the grafts are concerned with the building up of previously partially disintegrated structures to the condition typical of normal tail integument (Figs. 6–10). During this process the more internal or basal cells of the epidermis are either reduced in size or replaced by smaller cells during the early stages of regeneration. This process is later followed by a gradual dissimilation or breaking down of the basal layers of the epidermis, so that at approximately seventeen days following homeoplastic transplantation the resulting epidermis appears normal again. (See Fig. 10.) The stratum compactum shows no evidence of returning to normal until about the eleventh to fourteenth day. On the fourteenth day (Fig. 9) the fibers appear to be more or less condensed and are grouped into bundles. These loosely associated fibrous bundles contain a number of scattered epidermal cells in and around them. Subsequent observations made three days later (Fig. 10) show that the stratum compactum has assumed a wavy appearance and appears as a typical normal layer. Although the stratum compactum is only about one-third as thick as the corresponding layer in the surrounding back integument, the two unite

and form a perfect union. Usually a decrease in number of lymphocytes in the immediate region of the regenerating tissue can be noted. Finally, the integument at the end of 17 days appears to be normal as regards all histological features. However, while emphasizing the return to normal of the transplant as regards histological structure, it must be stated that the surface area remains the same throughout the entire process of regeneration. Thus the completely regenerated transplant in question is only approximately 50 per cent as large in actual surface area as the original autoplastic graft.

A comparison was also made of the process of regeneration in integument derived from tail region 1, as described above (50 per cent reduction), to similar transplants in which 18 per cent and 80 per cent reduction had occurred. In the transplants which had reduced but 18 per cent in area, the typical normal histological structure was observed in from 11 to 14 days, whereas in the case of grafts reduced 80 per cent in area, approximately twenty days were necessary for normal reconstitution to take place. This indicates, clearly, that the greater the degree of histolysis as based on surface area reduction, the longer the time necessary for complete histological regeneration.

Comparative Regenerative Rate of Integument from Four Tail Regions

In determining whether or not a susceptibility gradient to regeneration existed, it was necessary to make initial daily observations on the progress of histolysis of specific regional transplants, as well as subsequent histological examinations concerning the progress of regeneration. In these preliminary observations on tail-skin degeneration, the author was able to confirm previous work (Clausen, 1930) concerning an anterior-posterior gradient of susceptibility to histolysis. The earlier results were confirmed in that a regressive change in histolytic rate of tail integument from the most anterior region to the most posterior region of the tail was found to occur.

Daily macroscopical observations of the transplants from the four anterior-posterior regions of the tail revealed a decided difference in coloration during regeneration. It was noted that when graft number 1 had resumed its characteristic normal pigmentation at the end of 14 days following homeoplastic transplantation, graft number 4 remained apparently unchanged as regards coloration. Upon sectioning the transplants from regions 1 and 4, as well as those from the intermediate regions 2 and 3, a decided regional difference in the time taken for the grafts to complete the process of histological regeneration was noted (Table I). Table I presents the essential data obtained by the sectioning and making of a histological study of tail-skin transplants following their second or homeoplastic transplantation. However, it should be

noted that this table illustrates only differences in regeneration rate based on complete histological reconstitution of the transplants. Other histological examinations made at successive three-day intervals following homeoplastic transplantation show approximately the same variation in regenerative rate between transplants originally derived from the

TABLE I

Regeneration of Tail-skin Transplants [‡]

Regional Source *	Reduction in Area †	Time taken for return of normal histolytic picture			Average Time
		Case (1)	Case (2)	Case (3)	
	per cent	*days*	*days*	*days*	*days*
1	18	11	14	11	12
	50	14	17	17	16
	80	20	20	20	20
2	18	14	14	11	13
	50	17	17	20	18
	80	23	20	20	21
3	18	14	17	14	15
	50	20	23	20	21
	80	23	23	26	24
4	18	17	17	17	17
	50	23	23	20	22
	80	26	26	23	25

* Anterior-posterior level of tail from which transplant was originally obtained.

† Stage of histolysis (as determined by area reduction) at which second (homeoplastic) transplantation was made.

‡ To obtain sections of three transplants each from the four tail regions in three stages of histolysis which ultimately showed complete histological regeneration, it was necessary to section approximately one hundred and eighty transplants.

four tail regions. In other words, there is found to be a difference in regenerative rate between transplants 1, 2, 3, and 4 for the attainment of any of the various stages of regeneration. This gradient in regenerative rate was found to hold true regardless of whether the partially histolyzed transplants were placed on the back or tail regions of the host

animals. In other words, the particular transplantation site of any one transplant was of no consequence to the rate of regeneration occurring. The difference in regenerative rate for complete reconstitution between transplants 1 and 4 was approximately five days; transplants 2 and 3, as compared with transplant 1, being intermediate in this respect.

Similar observations on a group of individuals used as controls, where normal integument of the four tail regions was transplanted homeoplastically to the tail or back of normal larvæ, were also made. The results obtained from this series of experiments serve to indicate, clearly, that the transplantation procedure itself does not influence the histolysis or regeneration of the integument, either as regards the histological processes involved or the rate of regeneration which occurs.

Discussion

Histological studies made from sections of histolyzing tail integument as described in the present paper tend to show that different integumentary layers do not histolyze at the same rate. In other words, if the causal histolytic influence is the same throughout all regions of the transplant, it is evident that various layers of the integument differ as regards their susceptibility to histolysis. It may also be stated here that evidences of greater resorption of peripheral regions of the transplant as compared with more central areas is probably due to the fact that lymphocytes appear to be more numerous in the former regions and hence the histolyzed cellular elements are more quickly removed at such points.

In considering the coloration of transplants at various stages of histolysis, it may be concluded that changes in coloration are probably due to analogous changes in distribution of the underlying pigment cells. In this respect it may be stated that the number of pigment cells per any given area is always greater in histolyzing integument as compared with normal skin of the same region. It is also quite probable that variable changes in thickness of the epidermal portion of the graft during histolysis may be concerned with external changes in coloration. Since a considerable portion of the pigment material lies basal to the epidermal layer, any change in thickness of the latter results in a displacement of the former in respect to its distance from the cuticular surface of the integument. Consequently, the thinner the epidermal portion, as illustrated by degree of histolysis (Fig. 4), the nearer to the surface the pigment cells become arranged; and hence the greater the intensity of integumentary coloration, viewed externally. Conversely, in the 50 per cent reduction stage (Fig. 6) a less intense coloration is typical of

the more central portions of the graft. This condition is no doubt due to the fact that the greatly thickened epidermis forces the pigment layer an abnormal distance down and away from the surface of the integument.

The results of the present work indicate that there is an inherent difference in regenerative rate between integuments of different levels of the tail. Moreover, these results serve to emphasize that the rate of regeneration progressively increases from the posterior to the more anterior regions of the tail. Histologically, tail integument from various anterior-posterior tail regions appears to exhibit similar structural characteristics. On this basis, any morphological explanation for differences in regenerative rate can hardly be justified. It seems logical to assume, therefore, that the basis for such differences in regenerative rate must be a physiological one.

It is of interest to inquire at this time what factors operate to inhibit further histolysis of the homeoplastic transplants and thus permit regeneration to proceed. The results of the present and of previous work clearly indicate that histolysis is initiated by factors present in the blood stream only during larval metamorphosis. Assuming that regenerative tendencies are inherently present in the integument, we must then conclude that the histolytic effects are so powerful that any tendency towards regeneration is soon overcome and histolysis normally proceeds until the integument in question is completely destroyed. Upon transplantation, however, to a normal non-metamorphosing larva in which no histolytic blood factors are present, the integumentary transplant ceases to histolyze further. Concurrently, the inherent regenerative tendencies are no longer suppressed and the various histological elements are therefore capable of being regenerated.

The results of the present investigation seem to compare favorably with the earlier work of Morgan (1906) and Ellis (1908) in that the rate of regeneration increases from the distal to the more proximal regions of the tail. The results of Speidel (1929), however, indicate no change in regenerative rate of tail fin at various anterior-posterior levels. However, since it has been shown in the present paper that the regenerative rate differences between transplanted integument from various tail levels are not great and also since the work of Speidel concerned the regeneration of tail fin tissue *in situ*, it can readily be understood how the relatively small rate differences may have been masked by other physiological factors typical of the various tissues of the tail. The results of investigations by Comes (1928), which also possibly differ from the results as cited in this paper, may also be explained by the fact that his observations were concerned with regeneration of normal

tail fin tissue *in situ*. Furthermore, it may be well to state here that Speidel and Comes were both primarily concerned with linear growth of the tissue or tissues in question. Therefore, since the results as presented in the present paper are concerned chiefly with regeneration of partially histolyzed tissue and deal mainly with histological regeneration, a direct comparison with that of the earlier work can hardly be made.

The present work finally suggests the possibility that the underlying musculature of various tail regions may also exhibit differences in regenerative rate following partial histolysis. It is also of interest to inquire as to the possible physiological factors associated with such differences in regenerative rate. The difference in regenerative potentialities of integument at various levels is either an inherent quality present during early embryonic formation or one acquired during some stage of early larval growth. Work designed to adequately explain the above points is being carried on in this laboratory at the present time.

Summary and Conclusions

1. Uniform-sized tail-skin grafts were secured from extreme anterior and posterior regions and from two intermediate areas and transplanted, autoplastically, to the backs of *Rana pipiens* larvæ. Artificial metamorphosis was induced and the engrafted integument removed at certain stages of histolysis and transplanted, homeoplastically, to the back or tail of normal larvæ. Following homeoplastic transplantations, anterior grafts invariably regenerated, histologically, with greater rapidity as compared with posterior integumentary grafts. Intermediate tail-skin grafts regenerated at proportionate rates.

2. Control homeoplastic transplantations of skin from four regions of the tail, when grafted to the tail or back of normal larvæ, clearly indicated that histolysis does not occur in larval tail-skin following transplantation to normal individuals.

3. Histologically, the process of degeneration illustrates a definite, orderly sequence. Dissociation of various layers begins prior to the appearance of lymphocytes in the histolyzing area. Lymphocytes probably function chiefly as phagocytes.

4. The regenerative process is first evidenced, histologically, in the epidermal layers of the graft. The stratum compactum is shown to be the last layer of integument to completely regenerate.

5. It is concluded that an anterior-posterior gradient in regenerative rate is true for tail integument following partial histolysis. Apparently, differences in regenerative rate of integument at different levels of the

tail are inherent qualities. Possible physiological factors associated with such differences, however, are still undetermined.

LITERATURE CITED

CLAUSEN, H. J., 1930. Rate of Histolysis of Anuran Tail Skin and Muscle during Metamorphosis. *Biol. Bull.*, **59**: 199.

COMES, S., 1928. Sulla localizzazione delle potenze rigenerative del lembo cutaneo della coda nelle larve di Anfibi anuri. *Boll. Ist. Zool. R. Univ. Palermo.*, **1**: 180.

ELLIS, M. M., 1908. Some Notes on the Factors Controlling the Rate of Regeneration in Tadpoles of Rana clamita-Daudin. *Biol. Bull.*, **14**: 281.

ELLIS, M. M., 1909. The Relation of the Amount of Tail Regenerated to the Amount Removed in Tadpoles of Rana clamitans. *Jour. Exper. Zool.*, **7**: 421.

HELFF, O. M., 1926. Studies on Amphibian Metamorphosis. I. Formation of the opercular leg perforation in anuran larvae during metamorphosis. *Jour. Exper. Zool.*, **45**: 1.

HELFF, O. M., AND H. J. CLAUSEN, 1929. Studies on Amphibian Metamorphosis. V. The atrophy of anuran tail muscle during metamorphosis. *Physiol. Zool.*, **2**: 575.

LINDEMAN, V. F., 1929*a*. Integumentary Pigmentation in the Frog, Rana pipiens, during Metamorphosis, with Especial Reference to Tail-Skin Histolysis. *Physiol. Zool.*, **2**: 255.

LINDEMAN, V. F., 1929*b*. Reconstitution of Histolyzing Anuran Tail Skin following Homoplastic Transplantation. *Anat. Rec.*, **44**: 217.

MORGAN, T. H., 1906. The Physiology of Regeneraiton. *Jour. Exper. Zool.*, **3**: 457.

SPEIDEL, C. C., 1929. Studies of Hyperthyroidism. VI. Regenerative phenomena in thyroid-treated amphibian larvae. *Am. Jour. Anat.*, **43**: 103.

THE POLARITY OF THE EGG OF URECHIS CAUPO

ALBERT TYLER

(From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, and the William G. Kerckhoff Marine Laboratory, Corona del Mar, California)

It has been previously noted (Tyler, 1931) that the indentation of the unfertilized egg of *Urechis* marks the pole. This conclusion was based on observations of a small number of eggs in which the relation of the point of extrusion of the polar bodies to the indentation was followed. More recently Taylor (1931) reported some observations, made by Miss Vesta Holt on a slightly larger number of eggs, the results of which show no relation between the indentation and the point of extrusion of the polar bodies. This question has therefore been re-investigated on a larger scale.

The method of making such observations and the precautions to be taken have been previously described (Morgan and Tyler, 1930; Tyler, 1931). It consists of mounting a drop of eggs in sea water on a slide within a square of vaseline. The vaseline serves to support the cover-slip at a much greater distance above the slide than the diameter of the egg. It also serves to seal the preparation and thus prevent evaporation of the sea water from the drop. The drop makes contact with both the slide and the cover-slip but can be adjusted so as not to touch the vaseline. This last precaution, however, was probably unnecessary since the vaseline used was found to be non-toxic to the eggs. The eggs were fertilized in a dish and a sample transferred after one minute or less to the vaseline slide. They stay in position quite well on the vaseline slide, and the extra spermatozoa on the surface serve as markers by means of which any rotation of the egg can be detected. The eggs develop on the vaseline-slide into perfectly normal swimming larvæ of two or more days of age.

The indentation of the unfertilized *Urechis* egg is a concavity similar to that which would be produced by pushing in the surface of a rubber ball at one point to about forty-five per cent of the original diameter. The indentation disappears after fertilization, and, as has been elsewhere described (Tyler, 1932), the *Urechis* egg exhibits three types of behavior in regard to its rounding out. The egg may round out initially in three minutes after fertilization, become indented again at six minutes, and round out finally at ten minutes; or the indentation may not entirely disappear the first time; or the indentation may dis-

appear at three minutes and not reappear again. The eggs followed in the observations reported here were mainly of the first and third types. Eggs from freshly collected animals appear to be generally of the last type.

A total of 590 eggs were followed in the observations reported here. The results again show that the indentation marks the pole of the egg for the type that rounds out at three minutes and does not dent in again. For the type in which the indentation reappears this second indentation sometimes does not come back in the same place, but the point of extrusion of the polar bodies is almost invariably determined by the position of the second indentation.

Often eggs are obtained that have two indentations. The point of extrusion of the polar bodies usually coincides with one of the indentations. Such eggs generally lie with one of the indentations down so that they might be mistaken for eggs with only one indentation. This complication entered into some of the observations, as is evident from the results given in Table I.

TABLE I

Relation between Indentation and Point of Extrusion of Polar Bodies

Series A = isolated eggs with only one indentation.
Series B = random sample of eggs.

Divergence	Series A	Series B
0°–10°	92	72
10°–60°	12	20
60°–180°	10	35

In Table I are given the results of observations on freshly removed eggs from freshly collected animals. The eggs of Series A were all isolated and examined individually before being used in order to make sure that they had only one indentation. The results show 81 per cent of almost exact coincidence between the innermost central point of the indentation and the point of extrusion of the polar bodies; 11 per cent were close (10° to 60° divergence); and 8 per cent were 60° to 180° off. For the eggs of Series B no special precautions were taken to insure their possessing only one indentation, although they were obtained from batches of eggs most of which showed only one indentation. Out of 127 eggs in this series 35 were 60° to 180° off. But of these 35 eggs the position of the polar bodies was found to be below the egg. This result when compared with that of Series A may then be taken to mean that where coincidence was not obtained the unfertilized egg possessed another indentation lying below the egg and thus not visible. There is also the possibility as indicated below that in these cases the indentation reappeared in a different position, namely, on the underside of the egg,

but this is not likely since their sister eggs did not exhibit the reappearance of the indentation.

In Table II are given the results obtained on the type of egg in which a second indentation appears at six minutes after fertilization and then disappears at ten minutes. The point of extrusion of the polar bodies diverges by 60° to 180° from the central point of the first indentation in 41 per cent of the cases (117 eggs). But in 98 of these eggs the position of the second indentation was also noted and its central point was found to coincide with the point of extrusion of the polar bodies in 82 of the cases. Of the 130 cases of coincidence between the first indentation and the point of polar body extrusion, the position of the second indentation was noted in 87 cases and found to be identical with the first in 84 of them. In addition there were 66 eggs in which only the position of the second indentation was noted. The last column in Table II gives the results of all the observations in which the relation of the second indentation to the pole was noted. Here there are only 12 per cent of cases in which the divergence is very large.

TABLE II

Relation between indentation and point of extrusion of polar bodies. Eggs in which a second indentation appears.

Divergence	First Indentation	Second Indentation
0°–10°	130	210
10°–60°	36	12
60°–180°	117	29

It appears then that for this type of egg the position of the second indentation is a much better index of the pole of the egg than the first. This is particularly obvious in those cases in which the position of both indentations was observed—where the position of the polar bodies coincided with that of the first indentation it almost invariably coincided with the second, and where it did not coincide with the first indentation it coincided with the second in the great majority of the cases. However, even in the case of the first indentation there are 46 per cent of cases of coincidence (0° to 10° divergence), whereas a random distribution of the polar bodies with respect to this indentation would probably not give better than 8 per cent coincidence.

The main question involved in this work is whether or not the polarity of the *Urechis* egg is determined before fertilization, as appears to be the case for most animal eggs. For the type first discussed the results clearly show that polarity is already established in the unfertilized egg, and the pole is marked by the position of the indentation. For the

type in which a second indentation appears after fertilization there is no reason to doubt that polarity is established before fertilization although the position of the pole is not as clearly marked by the first indentation as by the one appearing after fertilization.

LITERATURE CITED

MORGAN, T. H., AND A. TYLER, 1930. The Point of Entrance of the Spermatozoön in Relation to the Orientation of the Embryo in Eggs with Spiral Cleavage. *Biol. Bull.*, **58**: 59.

TAYLOR, C. V., 1931. Polarity in Normal and Centrifuged Eggs of Urechis caupo Fisher and MacGinitie. *Physiol. Zool.*, **4**: 423.

TYLER, A., 1931. The Production of Normal Embryos by Artificial Parthenogenesis in the Echiuroid, Urechis. *Biol. Bull.*, **60**: 187.

TYLER, A., 1932. Changes in Volume and Surface of Urechis Eggs upon Fertilization. *Jour. Exper. Zoöl.* In press.

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